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# FAT EMBOLISM IN KOREAN BATTLE CASUALTIES

## ITS INCIDENCE, CLINICAL SIGNIFICANCE, AND PATHOLOGIC ASPECTS *

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The significance of fat embolism following trauma is controversial despite the large volume of literature on the subject. Some authors[1-4] have regarded fat embolism as a relatively common and serious complication of injury, while others[5-8] have believed that it is rarely severe enough to be of clinical importance. Surgeons who have handled large numbers of battle casualties in the Korean conflict[9,10] only exceptionally have been able to recognize the clinical syndromes described in the literature as characteristic of fat embolism. Because of such conflicting opinions, a study of this subject was begun in the Pathology Section of the 406th Medical General Laboratory. Necropsy cases of all types of trauma, including battle, were analyzed from a clinicopathologic viewpoint in an attempt to determine the specific significance of fat embolism among the many sequelae of serious injury. The present communication deals principally with 110 cases of fatal battle trauma received and reviewed at the laboratory during the year 1953. Data obtained from analysis of similar cases reviewed in 1952 and of cases of death following other types of trauma are presented whenever pertinent.

### MATERIAL AND METHODS

One hundred and thirty-two cases of fatal battle trauma were studied at our laboratory in 1953. Of these, 110 were selected for investigation of the incidence, clinical aspects, and pathologic changes of fat embolism. The remaining 22 were eliminated because clinical or pathologic data were inadequate or because death occurred later than 4 weeks after injury. This arbitrary time limit for exclusion of cases was selected because in our experience fat embolism has not

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been demonstrable beyond this time. Although a few of the soldiers in the series were killed instantly or died in the chain of medical evacuation, the great majority expired at Army hospitals where necropsy facilities were available. Thus, the 110 studied were not representative of all soldiers who died of battle wounds.

Forty-four of the necropsies were done by two trained pathologists at the 11th Evacuation Hospital, to which casualties developing post-traumatic renal failure were transferred. Thirty were performed at the 46th Army Surgical Hospital by a medical officer with previous training in Pathology. The remainder were done at numerous Army surgical, evacuation, and general hospitals in Korea and Japan by various medical officers, some of whom had little pathologic experience. Clinical abstracts were available for the great majority of patients necropsied at the 11th Evacuation Hospital and the 46th Army Surgical Hospital. The available clinical data on the rest of the patients were detailed in some instances, but in others, scant.

Routine necropsy sections of tissues fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin were available for study. In occasional cases, only several sections had been prepared; in the great majority, however, there were twenty or more. In 89 cases, oil-red-O fat stains were done on frozen sections of formalin-fixed lung tissue cut at 30 to 40 $\mu$ in thickness. In 33 cases, samples of kidney, and in 24, samples of one or several portions of the brain, were stained similarly. All oil-red-O sections were graded according to their content of intravascular fat. This grading was attended by some difficulty since the fat occasionally was distributed unevenly in a section. Because it was not feasible to do large numbers of fat stains on any one organ, estimation of the amount of intravascular vacuolation in routine hematoxylin and eosin sections (in which the fat is dissolved out) was resorted to as an ancillary method of grading.

The amount of fat in the lungs was graded 0 to 8, grade 1 representing less than 10 small emboli per section and grade 8 signifying the presence of emboli in a majority of high-power fields and the appearance of extensive beading of alveolar capillaries with globules of fat. The intermediate grades are difficult to define accurately and were selected on the basis of visual impressions. Since all sections were examined at least twice and were reviewed finally within a short period, it is believed that their relative grading was reasonably accurate. In sections of lung stained with oil-red-O and in those stained with hematoxylin and eosin, grades determined independently were identical in 47 per cent of cases; they differed by one in 33 per cent, by two in 17 per cent, and by three in 3 per cent of cases. When

grades differed by one, the higher of the two was selected for statistical purposes; when by two or three, the average was chosen.

The renal content of fat was graded 0 to 8, grade 1 corresponding to less than 5 emboli per section and grade 8, to the presence of multiple emboli in the great majority of glomeruli. Hematoxylin and eosin grading was less successful in the kidneys than in the lungs. However, it was of definite value, matching the oil-red-O grading closely with few exceptions.

So few brain sections contained fat that grading was not done. Rather it was considered more appropriate to describe the occasional positive sections as having minimal, slight, or moderate quantities of fat. Recognition of fat vacuoles in hematoxylin and eosin sections of brain was difficult, so that quantification of the fat without examination of oil-red-O stains was unreliable.

Independently of the microscopic study, the abstracts of the clinical records were combed with special attention devoted to duration of life, type and distribution of wounds, presence or absence of fractures, degree of shock, and prominence of cardiovascular, pulmonary, renal, and cerebral symptoms. Other pathologic findings at necropsy, especially those referable to the cardiovascular system and lungs, were tabulated in an attempt to discover if their presence could be related statistically to the amount of fat embolism observed microscopically.

## Review of the Literature

Exhaustive historical reviews of the literature on fat embolism have been written by Warthin[4] and more recently by Groskloss.[11] We shall summarize here only those aspects of the subject to which the present study may contribute pertinent information.

### *Etiology*

By and large, there is agreement that a significant degree of fat embolism is rare except as a sequel of injury to bone or adipose tissue.[3,4,11,12] The condition has been reported in a wide variety of non-traumatic disorders,[4,11] but with few exceptions it has been mild and without clinical significance in such cases. Types of injury to bone which can result in fat embolism include gross fracture, concussion or jarring without obvious fracture, and orthopedic operations and manipulations.[4,11] The most striking examples of fat embolism following trauma to adipose tissue are cases in which victims have been beaten with resultant extensive contusion of subcutaneous tissue.[13,14] In patients dying of extensive burns, fat embolism is common, but is very rarely of significant degree.[3,15]

*Pathogenesis*

Most authors have accepted Gauss's[16] theory of pathogenesis of post-traumatic fat embolism. He enumerated three conditions necessary for the entrance of free fat into the circulation: (1) rupture of the envelopes of the fat cells, (2) tearing of veins, and (3) increase in pressure locally, forcing the fat into the venous system. The pressure increase can be due to hemorrhage, edema, or other factors. In bone, anatomical fixation of the veins to the walls of the haversian canals is believed to prevent their collapse and to favor the admission of fat. In opposition to the theory of Gauss was Warthin's[4] observation that the total amount of fat in embolic form often far exceeds the fat content of the injured bone. Lehman and Moore[17] offered an explanation for this apparent paradox. They claimed that part of the "embolic" fat may be produced by conversion of endogenous emulsified fat in the serum into larger droplets. Using experimental animals, they were able to effect such a conversion with various chemical agents. There is no convincing evidence that an alteration in endogenous serum fat is a factor in human fat embolism, although such a possibility has not been excluded.

*Incidence*

Figures on the incidence of fat embolism vary greatly depending upon the types of cases analyzed and upon whether clinical or pathologic criteria are used to make the diagnosis. Wilson and Salisbury[8] stated that in a series of 1,000 consecutive battle casualties, 8 (0.8 per cent) showed clinical evidence of fat embolism. Newman[18] reported a 6 per cent clinical incidence in 89 consecutive patients with major injuries of the long bones. Using post-mortem criteria, Robb-Smith[3] diagnosed fat embolism in 81 per cent of 115 patients who suffered fatal accidents. Of 59 medical examiner's cases in which fracture of a lower extremity occurred, Vance[12] reported fat embolism in 75 per cent. Wyatt and Khoo[19] demonstrated fat emboli in every one of 30 patients dying after injury. Nineteen necropsy cases of trauma were studied by Denman and Gragg[1]; in 9 (47 per cent) there was fat embolism. Finally, fat emboli were found[20] in 34 of 51 battle casualties who were necropsied (67 per cent); in 10 of these the amount of embolic fat was "significant."

The incidence of fat embolism found at necropsy in non-traumatic cases is generally regarded to be low. Thus Robb-Smith[3] stated that in a "large series" of non-traumatic deaths, results were negative for fat embolism except in cases of burns, in which the degree was very slight. Vance[12] found fat emboli in only 7 of 82 cases of non-traumatic deaths. In each instance, the embolism was very mild. In 15 non-

traumatic sudden deaths, Wyatt and Khoo[19] were unable to demonstrate fat emboli. Of 22 of their patients dying after a lingering illness, only one showed emboli. This patient died 3 weeks after suffering extensive burns. Denman and Gragg[1] reported a 28 per cent incidence of fat embolism in 80 non-traumatic cases selected at random. In only one of these was the degree severe.

The incidence of systemic fat embolism has not been investigated as carefully as that of the pulmonary form. Vance[12] found fat emboli in the kidneys in only a few cases of "severe and fatal pulmonary fat embolism." Sheynis,[21] who reported an 88 per cent necropsy incidence of pulmonary fat embolism in traffic accidents with bone injuries, placed the incidence of systemic fat embolism at 12 per cent. Musselman *et al.*,[22] who approached the problem clinically by analyzing the urine for free fat, discovered that 52 per cent of 109 patients suffering moderate to severe injuries had lipuria on one or more of 15 consecutive daily examinations. On the other hand, only 12 per cent of 50 patients with non-traumatic illnesses showed lipuria on one of 7 consecutive daily examinations.

### *Significance*

The clinical significance of fat embolism is a subject on which many contradictory statements have appeared. For example, Gröndahl[6] stated that fat embolism is the cause of death in only 1 per cent of fatalities following fractures. In contrast, Warthin,[4] who encountered 9 deaths after fractures of long bones in a series of 560 necropsies, considered fat embolism the cause of death in all 9 instances. Denman and Gragg[1] considered that of their 19 necropsied patients who had suffered trauma, 6 (32 per cent) died of fat embolism. Robb-Smith[8] considered fat embolism a major cause of death in 25 per cent of 115 fatal accident cases examined at necropsy. In Vance's[12] series of 59 fatalities following fracture of a lower extremity, fat embolism was regarded as the primary cause of death in 3 (5 per cent). In 9 additional patients (15 per cent more), it was thought to be a significant factor in producing death. Musselman, Glas, and Grekin[22] concluded that fat embolism may be expected in about one half of all persons who have been either moderately or severely injured, and that about 10 per cent of patients having fat embolism will die of it.

### *Clinical Aspects*

There is almost universal agreement in the literature that fat embolism manifests itself clinically in at least two forms: pulmonary and cerebral.[4,11,12,23] It is stated that, in the pulmonary form, evidence of respiratory distress may appear immediately following injury[4,24] or

after a free interval of hours to days. Various signs and symptoms of respiratory embarrassment have been described. These include air-hunger, dyspnea, cyanosis, chest pain, cough, expectoration of thin mucoid sputum which may be blood-streaked, tachypnea, pulmonary edema, and râles. In some cases it is believed that the respiratory involvement is sufficiently extensive to cause death.

A syndrome of cerebral fat embolism more clear cut than the respiratory one has been described repeatedly in the literature. After a characteristic free interval of hours to days (3 to 6 days is said to be the average time[11]), the patient exhibits mental changes—insomnia or somnolence, apathy, amnesia, disorientation, delirium, stupor, and, finally, coma. A wide variety of neurologic disturbances, including paralysis, spasm, convulsions, rigidity, and reflex changes, may accompany the mental aberrations. One reported manifestation of cerebral fat embolism is failure to recover consciousness after operative anesthesia.[18] It has been stated that signs of increased intracranial pressure are almost always absent, and that the cerebrospinal fluid is normal in nearly every case.[25] Most of the reported cases of cerebral fat embolism have been fatal; however, recovery of patients with suggestive manifestations has been recorded a few times.[25,26] The cerebral form of fat embolism may or may not be preceded by respiratory signs or symptoms.[27]

Early authors[4] described, in addition to respiratory and cerebral forms, a cardiac syndrome for fat embolism characterized by precordial pain, a rapid, irregular heart rate, increasing venous pressure, and falling arterial pressure. Most modern writers, however, do not recognize the cardiac symptom complex.

Other signs of systemic fat embolism include: (1) the presence of free fat in the urine (this may be detected as early as the first day after injury[22]); (2) the appearance of crops of skin petechiae, most common on the neck, shoulders, and chest; and (3) the presence of intravascular fat globules[28] and perivascular edema and hemorrhage[29] in the eyegrounds. A final manifestation of fat embolism is an alteration of body temperature. In the typical case this is said to reach 103° F. at the height of the disease and to rise even higher terminally.[30]

### *Pathologic Features*

Pathologic changes due to fat embolism are encountered most commonly in the lungs,[11] which have been described as heavy and voluminous, with edema and patchy areas of emphysema. The pleural surfaces are stated to exhibit focal hemorrhages on a pink-gray background. The pulmonary arteries or veins may contain grossly visible fat. Micro-

scopically, the vessels, primarily arterial and capillary, are occupied and often distended by round, oval, dendritic, or stellate fat globules. When the fat droplets are numerous in the capillaries of the alveolar walls, these often have a beaded appearance. In a small number of cases bone marrow emboli also are found in the arterial lumina.[4] The alveoli may contain edema fluid, blood, and, in some instances, globules of fat.[23] The fat may be free or phagocytized by mononuclear or foreign body giant cells.[23]

Although emboli have been observed in almost every organ and tissue of the body when the fat has entered the systemic circulation, the pathologic changes in the systemic form of fat embolism are most intense in the brain, kidneys, and heart.[4,11] The brain characteristically shows crops of emboli in capillaries and arterioles of both gray and white matter. In severe cases, there are multiple petechiae and small areas of softening which are most numerous in the white matter of the cortex. The gray matter, with its richer blood supply, is much less vulnerable to necrosis.[12] Microscopically, one encounters small hemorrhagic and anemic infarcts often surrounding arterioles plugged with fat. The hemorrhagic infarct appears characteristically as an area of anemic necrosis surrounded by a ring of extravasated blood. The infarcts may excite an inflammatory response and finally evolve into glial scars.[25]

The kidneys show no gross changes. Microscopically, emboli are found chiefly in the glomerular capillaries, to a lesser extent in the intertubular vessels, and occasionally within the tubular lumina. When fat is abundant in the glomeruli, it forms characteristic pretzel-shaped masses. Necrotic or degenerative changes in the renal parenchyma secondary to fat embolism apparently do not occur.

The heart has been described by various authors[3,4,11] as showing right-sided dilatation in cases of pulmonary fat embolism. Where systemic embolism has involved the cardiac capillaries, the myocardium is said to exhibit small hemorrhages and areas of yellowish degeneration surrounded by hemorrhage.[4] Microscopically, fatty degeneration of the muscle fibers surrounding the emboli, hemorrhages, and anemic infarcts have been described.[4]

## Results of Analysis

### *Clinical Data*

The ages of the patients ranged from 18 to 32 years. The average age was 22; less than 10 per cent were over 25 years. Eighty-five per cent of the injured men were wounded by shell fragments; 15 per cent, by bullets. Ninety per cent of the shell fragment wounds and 33 per

cent of the bullet wounds were multiple. Three fourths of the patients had fractures. In a few instances, significant burns complicated the injuries. The anatomical distribution of the wounds is presented in Table I.

The duration of life after wounding ranged from seconds to 28 days. Approximately one fourth of the patients expired in less than 24 hours;

TABLE I

*Distribution of Wounds: 110 Cases*

| Location | Number of cases |
|---|---|
| Head and neck | 4 |
| Trunk | 22 |
| Mixed, excluding extremities | 5 |
| Extremities | 20 |
| Mixed, including extremities | 59 |

two fifths survived between 1 day and 1 week; and one third died later than 1 week after wounding.

## *Incidence*

Of the 89 patients from whom oil-red-O sections of lung were examined, 83 (93 per cent) showed pulmonary fat emboli. Using intravascular vacuolation in routine hematoxylin and eosin sections as the sole criterion for fat embolism, findings were positive in 87 of 110 cases, an incidence of 79 per cent. It is apparent, then, that minimal degrees of embolism occasionally escaped detection without the use of fat stains. Analysis of both routine and fat stains, as outlined in Material and Methods, revealed that 19 per cent of the soldiers showed moderate to marked degrees of pulmonary fat embolism (grades 5 to 7); 31 per cent showed slight degrees (grades 3 and 4); and 50 per cent showed minimal degrees or none at all (grades 0 to 2).

Table II shows the relation of pulmonary fat embolism to the time of death after wounding.

The more severe degrees of pulmonary fat embolism were seen most often in soldiers dying during the first week after wounding. The peak incidence was in the early part of the week after the first day. There was a definite tendency for the pulmonary fat content to be less during the second, third, and fourth weeks. We have seen fat embolism in soldiers who presumably lived no longer than a few minutes as well as in one who survived 24 days after injury.

Extensive experience with both routine and fat stains has shown that systemic fat emboli are most abundant and easiest to recognize

microscopically in the kidneys. For this reason, the presence of fat in renal vessels has been regarded as the most sensitive pathologic index of the entrance of emboli into the systemic circulation. Previous studies of workers at this laboratory[14,31] and of others[12] have revealed that (1) renal involvement rises in incidence and grade with increasing degrees of pulmonary fat embolism, and (2) minimal to slight

TABLE II

*Relation of Fat Embolism to Duration of Life after Wounding*

| | 0-1 day | 1+-4 days | 4+-7 days | 7+-14 days | 14+-30 days |
|---|---|---|---|---|---|
| Number of cases | 26 | 24 | 22 | 26 | 9 |
| Average grade | 2.5 | 3.4 | 2.9 | 2.2 | 2.0 |
| Percentage of cases, moderate to marked | 19 | 33 | 23 | 12 | 0 |

degrees (grades 0 to 4) of the latter are only rarely accompanied by the appearance of fat in the kidneys. For these reasons, in the present series, renal fat stains were done routinely only in cases in which the grade of pulmonary fat embolism was 4 or higher. In 26 such instances, the kidneys were positive in 17. In the remaining cases, in which the grade of pulmonary fat embolism was 3 or less, and in which hematoxylin and eosin sections of the kidneys disclosed no evidence of fat embolism, it was considered justifiable and conservative to grade the renal fat embolism as "none to minimal" (grades 0 to 2). Combining the results of grading both routine and fat stains, it was found that of the 110 patients, 92 per cent showed minimal or no renal fat embolism. Four per cent exhibited slight (grades 3 and 4), and an additional 4 per cent, moderate to marked degrees (grades 5 to 7).

Because of the importance of cerebral fat embolism as a potential cause of death, sections of the central nervous system were studied with fat stains whenever the pulmonary grade was 4 or greater, and brain tissue was available. Usually one section of cortex was examined. Often additional sections from cortex and other parts of the brain were studied as well. In the 24 cases in which the brain was stained with oil-red-O, findings were positive in 5. Cerebral fat embolism was less common and almost always less marked in degree than renal fat embolism. Thus, of the 24 cases mentioned, the kidney was positive for fat in 15, an incidence three times that of the brain. Similarly, in a previous series dealing with various types of trauma,[14] of 42 cases in which both renal and cerebral fat stains were done, the kidney was positive for emboli in 18 and the brain in only 9. In no case, in either the present or the previous series, was the brain posi-

tive when the kidney was negative. In the present series, in 3 of 4 cases in which renal fat embolism was slight, cerebral embolism was minimal in 2 and absent in the third (in the fourth case no oil-red-O section of brain was available). In 3 of 4 cases in which renal fat embolism was moderate to marked, brain embolism was minimal in 2 and moderate in the third (in the fourth case no oil-red-O section of brain was available).

In summary, about 90 per cent of soldiers dying in hospitals less than 1 month after battle trauma showed pulmonary fat embolism at necropsy; however, only 19 per cent showed more than a slight degree. Less than 10 per cent of the soldiers had more than minimal renal fat embolism, and much fewer had more than minimal cerebral involvement.

With regard to the comparative incidence and severity of fat embolism in various types of trauma, our experience has been that the highest incidence and most severe grades have been observed in individuals who have been severely beaten and who had extensive contusions of the extremities. We have seen 13 such cases. A study of over 50 deaths following vehicular accidents has revealed an incidence and severity of fat embolism roughly paralleling that complicating battle trauma. The lowest incidence and mildest degrees of pulmonary fat embolism among the large groups of traumatic cases studied have been in patients who have died of single bullet wounds of the head or chest (over 40 cases examined).

Although we have not made an organized study of the incidence of pulmonary fat embolism in non-traumatic cases, we have been impressed with both its rarity and its mildness whenever present.

In 18 patients dying after extensive burns, we have found absent or minimal pulmonary fat embolism in 15, slight embolism in 2, and moderate embolism in one.

### *Pathogenesis*

Little new information on the pathogenesis of fat embolism was obtained from an analysis of the present series of cases. It was not possible to estimate accurately from the necropsy records the amounts of injury to soft tissues and bone, so these could not be related to the degree of pulmonary fat embolism. However, it was feasible to make a correlation between the topography of the wounds, the presence or absence of fractures, and the amount of fat in the lungs. Thus, it was found that of 31 patients having wounds confined to the head, neck, trunk, or a combination of these, only one showed moderate pulmonary fat embolism. In contrast, of 79 patients whose extremities were

involved, 20 showed moderate to marked fat embolism. One explanation for this difference probably depends upon the greater amount of osseous damage suffered in the 79 cases involving the extremities. Of the latter, 63 were associated with fractures and 19 of these showed moderate to marked pulmonary fat embolism. On the other hand, only one of the 16 patients without accompanying fractures exhibited moderate embolism. Although the wounded-in-action cases provided no opportunity for correlation between the amount of soft tissue damage and the degree of fat embolism, study of our 13 cases in which death was due to beating suggested the importance of trauma to adipose tissue in the production of fat embolism. These patients at necropsy commonly showed marked pulmonary, and occasionally marked systemic fat embolism despite the absence of obvious fractures. It is possible, of course, that jarring of the long bones with microscopic fractures provided a source of embolic fat in these cases.

### *Clinical Aspects*

An attempt to delineate a pulmonary symptom-complex in the present series of cases was unsuccessful. Dyspnea and cyanosis often were mentioned as prominent features in the courses of these patients. However, there appeared to be no correlation between these symptoms and the amount of fat in the lungs. Admittedly, a rare patient had at some interval after wounding a more or less acute onset of respiratory distress, and died, showing moderate to marked pulmonary fat embolism. On the other hand, such histories were obtained also in association with only minor degrees of embolism, and usually were absent in cases showing high grades.

Likewise there was no evidence in this series to support a cardiovascular symptom-complex. No signs of right-sided heart failure which could be correlated with moderate or marked pulmonary fat embolism were observed. In a small percentage of the cases, in which it was possible to estimate the degree of shock, no correlation was demonstrable between it and the pulmonary fat content. Furthermore, the occasional occurrence of unexpected sudden death bore no consistent relationship to the quantity of embolic fat in the lungs.

With reference to systemic fat embolism, ophthalmoscopic examinations and laboratory examinations of the urine for fat were not recorded in our abstracts. Cutaneous petechiae were noted in occasional cases, but could not be correlated statistically with the degree of fat embolism at necropsy.

Only two patients presented a clinical picture suggestive of cerebral fat embolism. At necropsy, one had a moderate degree, while the other

showed cerebral edema, but no fat embolism. The former (case 1) was a 29-year-old soldier who sustained many shell fragment wounds of the extremities causing numerous fractures. Twenty-four hours after extensive débridement and multiple amputations, he became stuporous and hypertensive. Cranial exploration revealed the brain to be under considerable pressure, but there was no hemorrhage. The patient expired 10 hours after his second operation (approximately 52 hours after wounding).

In our 1952 series of soldiers dying after battle wounds,[14] there was a similar incidence of clinically apparent cerebral fat embolism (1 of 109 patients). This one patient (case 2), who sustained many wounds from a land mine explosion, lapsed into deep coma following a leg amputation and extensive débridement of wounds. Pupillary signs, generalized convulsions, and hypertension appeared 49 hours post-operatively. Cranial exploration gave negative findings. Skin petechiae appeared and the patient died 55 hours after his original operation (63 hours after wounding).

### *Pathologic Features*

We have been unable to find any characteristic gross pathologic changes in lungs showing fat embolism. There was no edema, focal hemorrhage, patchy emphysema, or atelectasis described consistently in the lungs with the more severe grades of embolism. Table III shows the average combined weights of 102 pairs of lungs correlated with

TABLE III

*Average Combined Weights of the Lungs in Fat Embolism*

| Grade of embolism | Time of death | | | |
|---|---|---|---|---|
| | Under 1 day | 1–2.5 days | 2.5–5 days | Over 5 days |
| | gm. | gm. | gm. | gm. |
| 0–2 | 1246 (12 cases) | 963 (4) | 1300 (8) | 1171 (29) |
| 3–4 | 1404 (7) | 1020 (2) | 1064 (8) | 1135 (14) |
| 5–7 | 871 (5) | 931 (4) | 1235 (3) | 1058 (6) |

the grades of fat embolism at various times at which death occurred following wounding. These data afford no support for the thesis that fat embolism favors the development of pulmonary edema.

After considerable experience it became possible to recognize with ease the vacuoles produced by fat emboli in hematoxylin and eosin sections of the lung. In the larger vessels these appeared as large,

round or oval spaces which often coalesced (Fig. 1). Unlike artifactitious spaces, which merely displaced intravascular red cells, the vacuoles of fat compressed them so that their surfaces became concave along the margins of the vacuoles. Arterioles and capillaries were plugged and distended by round, oval, or reniform vacuoles. At the periphery the vessel walls and their endothelial linings were tightly compressed, suggesting great tension (Fig. 1). In sections of tissues fixed in acid formalin the deposition of delicate, golden-yellow crystals of formalin pigment in the vacuoles was of confirmatory value in identifying them as fat. The use of fat stains revealed a striking picture often outlining the pulmonary arterial tree and capillaries of the alveolar walls (Fig. 2).

In 4 of our 110 cases bone marrow emboli were found in small numbers in the pulmonary arteries (Fig. 3). In one case a tiny fragment of embolic skin was seen. Commonly, thrombi composed largely of fibrin and eventually penetrated by endothelial cells lay in juxtaposition to the embolic fat globules. Since these thrombi, more often than not, were situated away from the fat emboli, it seemed probable that the association of the two was of a collision type.

Microscopically, just as grossly, we were unable to correlate the presence of pulmonary fat emboli with focal edema, hemorrhage, emphysema, or atelectasis.

The presence of fat droplets within the alveoli was unusual. Occasionally, they were extravasated with blood. In one case there was focal massive outpouring of fat into the air spaces in the absence of obvious hemorrhage. Phagocytosis of extravasated fat by alveolar phagocytes was not observed. The lipochrome granules, so commonly seen in these cells, stained orange or rust color with oil-red-O in contrast to the bright red of the embolic fat. Likewise, the quantity of phagocytes bore no consistent relationship to the amount of fat within nearby vessels. In only one case was there a reaction of vascular endothelium to fat emboli. In it the endothelial cells surrounding the fat were filled with numerous, small, uniform vacuoles, presumably fatty. No variation in the appearance of the emboli was detected in relation to time. The fat seen in several cases in which death occurred less than 10 hours after wounding appeared identical to that observed within pulmonary vessels as late as 24 days after injury.

No clues were apparent from the histologic sections of lung as to how the embolic fat was eliminated. It is possible that local enzyme activity is responsible for the gradual elimination of the pulmonary fat or that the droplets are continually being propelled into the systemic circulation and excreted by the kidneys. The failure to find fat

regularly in the air spaces does not exclude the possibility of rapid elimination in the sputum.

The topographic localization of fat emboli within the lungs was not worked out in detail. Generally, sections taken from various regions of the lungs contained fairly similar amounts of fat. Occasionally, one section differed by two grades from another, and rarely, by three. In sections in which only a small amount of fat was present, it often was confined to a thin rim of lung beneath the pleural surface.

When more than minimal, renal fat embolism usually was easy to recognize in hematoxylin and eosin sections by observing the vacuolation of capillaries of the glomerular tuft (Fig. 4). When the degree of embolism was minor, the fat was most likely to appear in the glomeruli near the renal capsule. Emboli were seen also in the intertubular vessels, chiefly in the cortex (Fig. 5). Rarely, fat in the form of droplets or casts was visible within the tubules (Fig. 6). In a single case of death due to beating, fibrin thrombi lay in juxtaposition to the fat emboli in the glomeruli. Fat emboli were seen in the kidneys as early as 10 to 18 hours after wounding and as late as 17 days.

In the central nervous system, emboli scattered in groups were seen in both gray and white matter. Vacuolation in hematoxylin and eosin sections of brain often was difficult to recognize. Occasionally, an empty dilated vessel with a tenuous wall was highly suggestive of fat embolism (Fig. 7). In only 2 cases, cases 1 and 2, have we observed parenchymal lesions secondary to the emboli. In those, petechiae and small areas of anemic necrosis (Fig. 8) appeared, especially in the white matter of the cortex, but elsewhere as well. The infarcts were characterized by loosening and loss of tinctorial properties of the intercellular framework, and shrinkage of both nerve and glial cells. Affinity for both eosin and fat stains was decreased. Some lesions showed infiltration by polymorphonuclear leukocytes. In occasional infarcts, centrally situated capillaries or arterioles were plugged with fat. Using fat stains, emboli were seen most commonly in the gray matter of the cortex (Fig. 9). A confusing element in the interpretation of fat stains of the brain was the frequent presence, in a perivascular location, of phagocytes containing lipochrome granules. These granules, which had a golden color in hematoxylin and eosin sections, seemed to be entirely unrelated to fat emboli, ofter appearing in their absence.

Oil-red-O stains were done on only a few heart sections. In case 1, small groups of capillaries were distended with fat globules (Fig. 10). The myocardium showed a few, scattered, small foci of degeneration, characterized by hyalinization of sarcoplasm and nuclear pyknosis. It

was not certain that these changes were ante mortem. Neither fatty degeneration of muscle fibers surrounding the emboli nor petechiae were observed.

Next to the lungs and kidneys, fat emboli were recognized most easily in hematoxylin and eosin sections of the adrenal glands and spleen. There they produced characteristic round, oval, and sausage-shaped vacuoles which were most numerous in the subcapsular zones (Figs. 11 and 12). In occasional cases, vacuoles highly suggestive of fat emboli were seen in other organs. With the exception of the brain, we have not seen degenerative parenchymal lesions secondary to fat embolism.

## Discussion

Because of the many variables existing in any large series of traumatic cases, our high incidence of fat embolism (about 90 per cent) cannot be compared reasonably with incidences in most other reported series. Mallory's[20] collection of necropsies on World War II wounded is perhaps as close in nature to ours as any in the literature. His incidence of pulmonary fat embolism (67 per cent) is lower than ours. However, his figure for "significant" embolism (20 per cent) tallies closely with ours for moderate to marked embolism (19 per cent). Likewise, our incidence of moderate to marked systemic fat embolism (4 per cent) is identical to that given by Mallory for "significant" systemic fat embolism.

The major disagreement we have with the widely accepted concept of fat embolism as a clinicopathologic entity is in regard to its rôle in producing significant pulmonary dysfunction. Analysis of our cases did not reveal statistical evidence that fat embolism causes pulmonary distress. Likewise, there was no pathologic correlation between the amount of pulmonary fat embolism observed and the presence and degree of pulmonary edema, pulmonary hemorrhage, atelectasis, emphysema, or right-sided cardiac dilatation. Mallory[20] was similarly unable to correlate fat embolism and pulmonary edema on a statistical basis. While such evidence cannot exclude the possibility that serious pulmonary embarrassment may result from embolic fat in occasional cases, such cases must be quite uncommon, if they exist at all, in order to escape detection in such a large series as the present one.

In reviewing the literature on fat embolism, we have been impressed by the lack of "controls," i.e., injured patients who died showing no, or at most slight, fat embolism at necropsy. Failure to compare such cases with cases of moderate to marked fat embolism exposes one to the risk of fallacious reasoning in implicating fat embolism as the cause of various clinical and pathologic findings. Again, the frequent

absence of pulmonary symptoms and signs antedating the onset of cerebral fat embolism[11,25,29] is difficult to explain if one holds that pulmonary fat embolism produces respiratory embarrassment. Cerebral fat embolism is, with possible rare exceptions, encountered only when the degree of pulmonary involvement is moderate to severe.

Our conclusions, based on clinical data, are similar to those of Armin and Grant,[32] who employed experimental methods. These authors produced pulmonary fat embolism by injecting rabbit perirenal fat into ear veins of unanesthetized rabbits. They estimated the amount of fat which it was necessary to inject in order to produce a degree of pulmonary fat embolism equivalent to a severe degree in the human. This estimate was based on measurement of the extractable fat content of severely involved human and rabbit lungs, as well as on grading of histologic sections. Their conclusions were: (1) The production of pulmonary fat embolism corresponding to a severe grade in the human caused no change in blood pressure, pulse rate, or respiration in the normal rabbit. The dose given to produce such embolism was much less than that required to kill the rabbit. (2) In animals subjected to severe hemorrhage (averaging about 40 per cent of the blood volume) the additional production of severe pulmonary fat embolism did not alter the clinical picture or increase the mortality above that due to hemorrhage alone. (3) The production of severe pulmonary fat embolism had no effect on the response of the animals to transfusion after hemorrhage. (4) Unless the rabbit tolerates fat embolism better than man, human gross pulmonary fat embolism is unlikely to provoke symptoms or to be alone or in part responsible for death after injury.

As a corollary to our inability to relate evidence of pulmonary distress to fat embolism, our mortality rate (i.e., the percentage of our traumatic deaths attributable to fat embolism) is exceedingly low (about 1 per cent) as compared with rates given elsewhere in the literature. We have considered as proved fatal cases only those in which death was associated with a typical cerebral syndrome clinically and a significant degree of cerebral fat embolism pathologically. The high mortality rates given in the literature depend largely upon the assumption that moderate to severe grades of pulmonary fat embolism, whenever demonstrated, represent a primary cause of death or contribute to death as a major factor. Since we have been unable to correlate pulmonary dysfunction with fat embolism, we must consider this assumption to be gratuitous.

Several objections may be raised to the nature of the material from

which we have drawn our conclusions. (1) The clinical abstracts at our disposal were not invariably detailed. (2) Field surgeons caring for large numbers of wounded soldiers, most of them seriously ill, may not have recorded accurately or uniformly any but the more severe signs or symptoms. (3) Pathologically, we were unable to judge the ages of pulmonary fat emboli; that is, we could not determine whether the emboli had accumulated in successive crops over a period of time or had involved the lungs in a single massive onslaught. Such a determination, if possible, would undoubtedly have increased the value of our clinicopathologic correlation. Despite these objections (one or more of which apply equally well to other series in the literature), we think that: (1) Little in the way of conclusive proof has been offered for the opinion that moderate to severe pulmonary fat embolism causes significant pulmonary dysfunction or death; (2) other possible sequelae of injury have not been excluded adequately as causative agents of pulmonary signs and symptoms when such do appear.

With regard to cerebral fat embolism, we have been impressed with the rarity with which it is present in significant amounts (1 per cent of necropsied cases of battle trauma).

## Conclusions

A clinicopathologic analysis of 110 cases of death occurring at military hospitals up to 4 weeks after battle trauma was made to determine the incidence and significance of fat embolism.

Fat embolism, as evidenced by the presence of fat droplets in the pulmonary vessels, was demonstrable in approximately 90 per cent of the 110 patients. In only 19 per cent was the degree of pulmonary fat embolism more than slight.

Of the 110 patients, only 4 per cent showed more than slight systemic fat embolism, as evidenced by the appearance of fat in the kidneys, and only 1 per cent showed fatal (cerebral) fat embolism.

From both clinical and pathologic viewpoints, there was no evidence from the analysis that fat in the lungs causes pulmonary dysfunction or death. This finding is at marked variance with the concept of the significance of pulmonary fat embolism accepted in the literature.

Fat emboli may be recognized and quantified with considerable accuracy without the use of fat stains by searching for and estimating the amount of intravascular vacuolation. This can be done with greatest success in the lungs, and with somewhat less success in the kidneys.

It is possible that detailed clinical and laboratory investigation of

injured patients may uncover a significance of fat embolism which is hidden by the more obtrusive complications of trauma in a routine clinical investigation.

I acknowledge the contributions of all medical officers who cared for the soldiers forming the basis of this investigation. Special gratitude is due Maj. John Lukeman, who initiated the study; 1st Lt. Joseph Strawitz and Capt. William Blake, who performed many of the necropsies; and members of the Surgical Research Team, who studied many of the patients clinically.

## REFERENCES

1. Denman, F. R., and Gragg, L. Fat embolism: a diagnostic enigma. *Arch. Surg.*, 1948, **57**, 325–332.
2. Glas, W. W.; Grekin, T. D., and Musselman, M. M. Fat embolism. *Am. J. Surg.*, 1953, **85**, 363–369.
3. Robb-Smith, A. H. T. Pulmonary fat-embolism. *Lancet*, 1941, **1**, 135–141.
4. Warthin, A. S. Traumatic lipaemia and fatty embolism. *Internat. Clin.*, 1913, s. 23, **4**, 171–227.
5. Darrach, W. Discussion of: Harris, R. I.; Perrett, T. S., and MacLachlin, A. Fat embolism. *Ann. Surg.*, 1939, **110**, 1113–1114.
6. Gröndahl, N. B. Untersuchungen über Fettembolie. *Deutsche Ztschr. f. Chir.*, 1911, **111**, 56–124. (Cited by Scuderi.[80])
7. Whitson, R. O. A critique of fat embolism. *J. Bone & Joint Surg.*, 1951, **33-A**, 447–450.
8. Wilson, J. V., and Salisbury, C. V. Fat embolism in war surgery. *Brit. J. Surg.*, 1943–44, **31**, 384–392.
9. Artz, C. P. Personal communication.
10. Howard, J. M. Personal communication.
11. Groskloss, H. H. Fat embolism. *Yale J. Biol. & Med.*, 1935–36, **8**, 59–91, 175–197, 297–315.
12. Vance, B. M. The significance of fat embolism. *Arch. Surg.*, 1931, **23**, 426–465.
13. Bürger, L. Die Fettembolie und ihre Bedeutung als Todes-und-Krankheitsursache. *Vrtljschr. f. gerichtl. Med.*, 1910, **39**, Suppl., 159–172. (Cited by Vance.[12])
14. Scully, R. E. Fat embolism. In: Annual Historical Report, 406th Medical General Laboratory, 1952, pp. 197–201.
15. Gröndahl, N. B. Cited by Groskloss.[11]
16. Gauss, H. The pathology of fat embolism. *Arch. Surg.*, 1924, **9**, 592–605. (Cited by Vance.[12])
17. Lehman, E. P., and Moore, R. M. Fat embolism, including experimental production without trauma. *Arch. Surg.*, 1927, **14**, 621–662.
18. Newman, P. H. The clinical diagnosis of fat embolism. *J. Bone & Joint Surg.*, 1948, **30-B**, 290–297.
19. Wyatt, J. P., and Khoo, P. Fat embolism in trauma. *Am. J. Clin. Path.*, 1950, **20**, 637–640.
20. [Mallory, T. B.] General Pathology of Traumatic Shock. In: The Physiologic Effects of Wounds, by the Board for the Study of the Severely Wounded. North African-Mediterranean Theater of Operations. Office of the Surgeon General, Department of the Army, Washington, D.C., 1952, pp. 283–305.

21. Sheynis, M. I. Ukranian Inst. for Orthop. and Traumatol., Kieff. [Traumatic fat embolism.] *Arkh. Patol.*, 1951, **13**, Pt. 4, 103–104. (Abstracted in Excerpta Medica, General Path. & Path. Anat., 1952, **5**, 517.)

22. Musselman, M. M.; Glas, W. W., and Grekin, T. D. Fat embolism, a clinical investigation. *A. M. A. Arch. Surg.*, 1952, **65**, 551–556.

23. Warren, S. Fat embolism. *Am. J. Path.*, 1946, **22**, 69–87.

24. Bürger, L. Die Bedeutung der Fettembolie für den Kriegschirurgen. *Med. Klin.*, 1915, **11**, 996–1001. (Cited by Warren.[23])

25. Winkelman, N. W. Cerebral fat embolism; a clinicopathologic study of two cases. *Arch. Neurol. & Psychiat.*, 1942, **47**, 57–76.

26. James, E. S. Fat embolism. *Canad. M. A. J.*, 1950, **62**, 548–550.

27. Strauss, H. Cerebrale Fettembolie. *Zentralbl. f. d. ges. Neurol. u. Psychiat.*, 1933, **66**, 385–400. (Cited by Groskloss.[11])

28. Oppenheimer, H. Multiple Fettembolien des grossen Kreislaufs. *Klin. Wchnschr.*, 1929, **24**, 25. (Cited by Newman.[18])

29. McArdle, M. J. F. Personal communication. (Cited by Newman.[18])

30. Scuderi, C. S. Fat embolism, a clinical and experimental study. *Surg., Gynec. & Obst.*, 1941, **72**, 732–746.

31. Lukeman, J. Fat embolism. In: Annual Historical Report, 406th Medical General Laboratory, 1951, pp. 92–94.

32. Armin, J., and Grant, R. T. Observations on gross pulmonary fat embolism in man and the rabbit. *Clin. Sc.*, 1951, **10**, 441–469.

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[ *Illustrations follow* ]

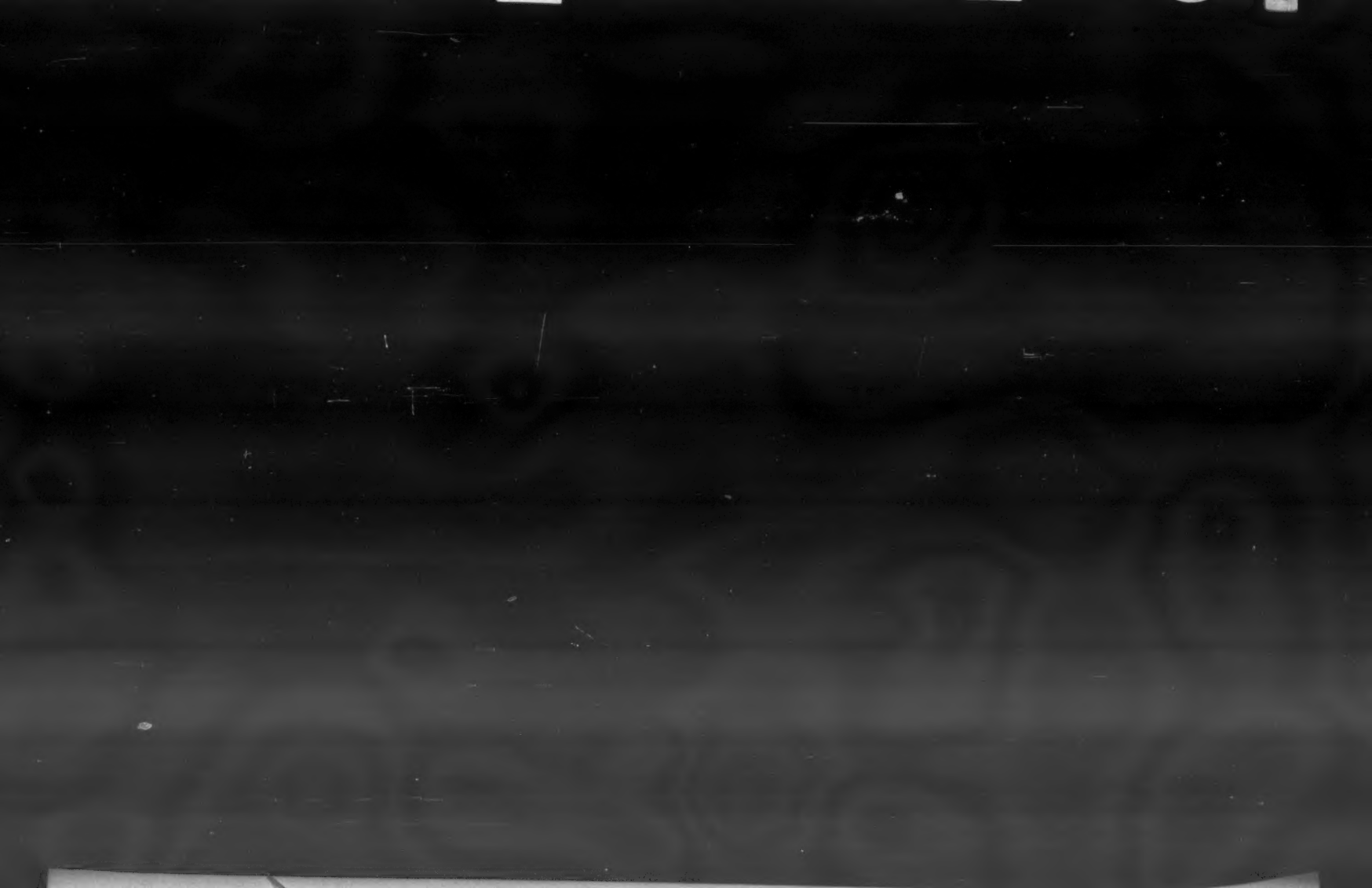
## LEGENDS FOR FIGURES

FIG. 1. Lung. The arteries contain multiple round and oval vacuoles. Several capillaries of the alveolar walls are distended by large vacuoles. Hematoxylin and eosin stain. × 110.

FIG. 2. Lung. Arteries and capillaries of the alveolar walls are plugged with fat. The alveolar walls present a beaded appearance. Oil-red-O stain. × 100.

FIG. 3. Lung. Bone marrow embolus in artery. Hematoxylin and eosin stain. × 200.

FIG. 4. Kidney. Many capillaries of the glomerular tufts are distended by fat vacuoles. Hematoxylin and eosin stain. × 100.



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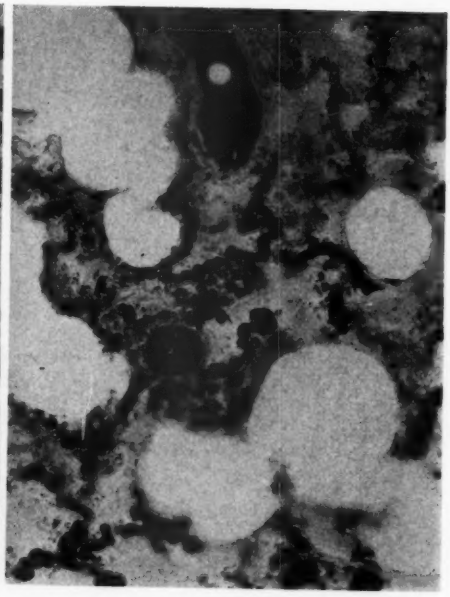

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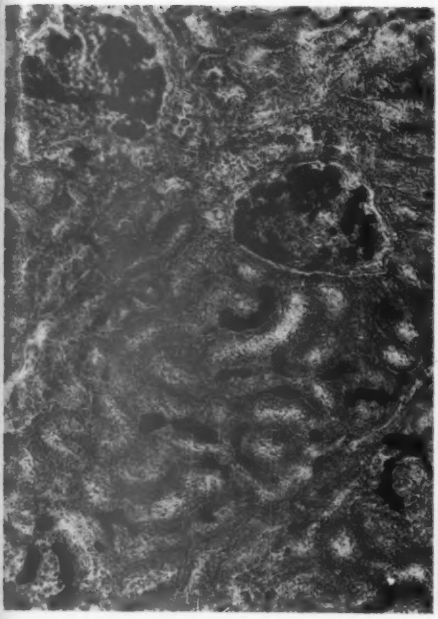
FIG. 5. Kidney. The glomeruli contain pretzel-like masses of fat. The intertubular vessels also are plugged with fat. Oil-red-O stain. × 100.

FIG. 6. Kidney. The uniformly black intratubular casts are fat. The paler granular casts are of hemoglobin type. Oil-red-O stain. × 100.

FIG. 7. Posterior pituitary lobe. Three small vessels are distended by vacuoles suggestive of fat. Hematoxylin and eosin stain. × 650.

FIG. 8. Cerebral cortex. A pale, irregular area of rarefaction is seen at the junction of gray and white matter. Oil-red-O stain. × 50.



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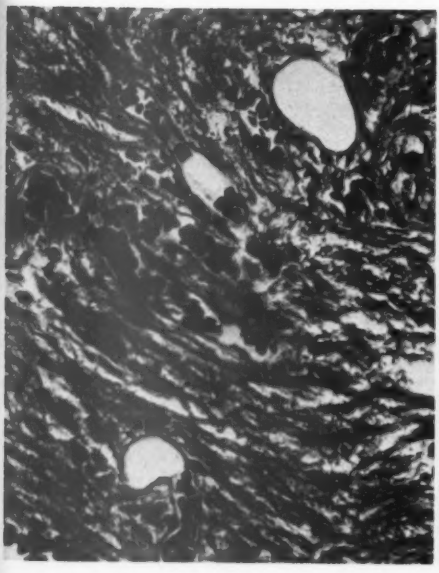

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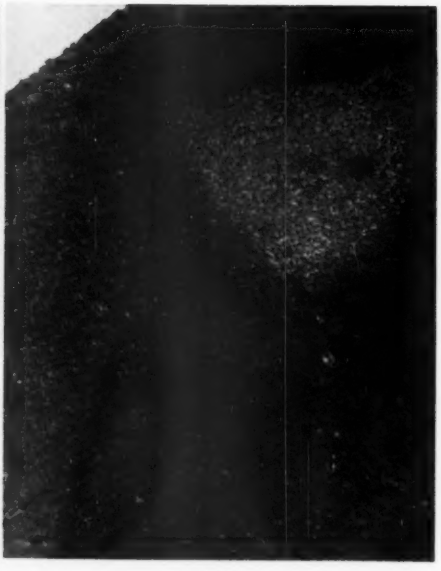

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FIG. 9. Cerebral cortex. The gray matter contains numerous fat emboli. Oil-red-O stain. × 100.

FIG. 10. Myocardium. A group of capillaries contain fat emboli. Oil-red-O stain. × 108.

FIG. 11. Adrenal cortex. Many sinusoids are distended by round and sausage-shaped vacuoles produced by fat emboli. Hematoxylin and eosin stain. × 138.

FIG. 12. Spleen. Several arterioles are distended by vacuoles of fat. Hematoxylin and eosin stain. × 100.

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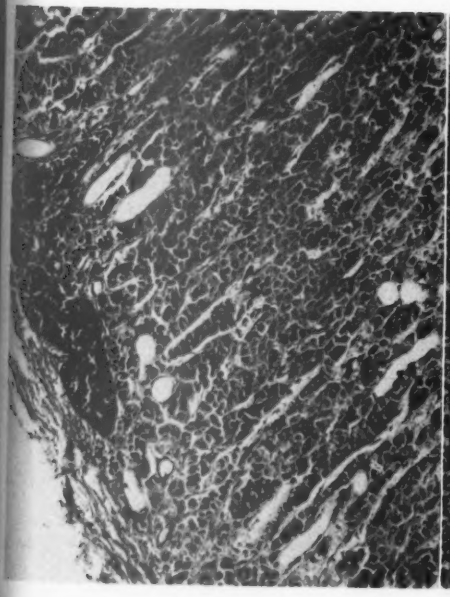

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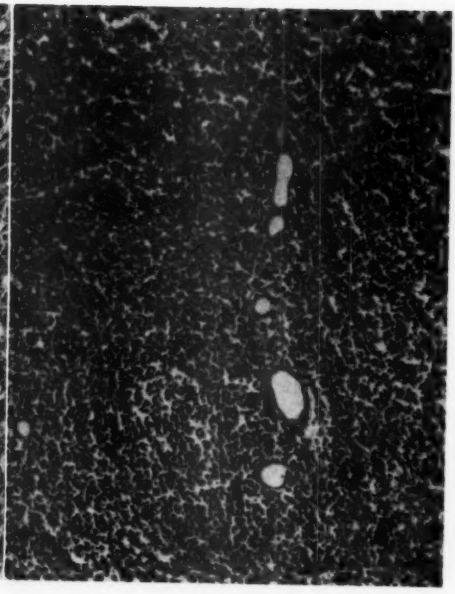

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# EMBOLI OF CEREBRAL TISSUE IN THE LUNGS FOLLOWING SEVERE HEAD INJURY *

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Emboli of tissue fragments have been found occasionally in the lungs at necropsy. Cases have been reported of emboli of the bone marrow (Fisher[1]), liver tissue (Straus[2]), and brain (Krakower[3] and Oppenheimer[4]). The present paper is concerned with a report of 4 cases in which multiple emboli of brain tissue were observed microscopically in the lungs following severe head injury.

## Report of Cases

### *Case 1*

W. T., a white male, 71 years of age, died 2 hours after a fall from a ladder. Necropsy (no. 7196) was performed 3 hours post mortem and revealed a linear fracture of the base of the skull with subdural and subarachnoid hemorrhages. The brain showed contusions externally and localized intracerebral hemorrhages on section. There were laceration and hemorrhage in the pons. The lungs were of usual weight and showed no gross lesions. Microscopically, there was laceration of the brain stem with extensive destruction of tissue. Examination of random sections of the lungs showed distention of several peripheral pulmonary arterioles by emboli of cerebral tissue (Fig. 1). Neurons, glial cells, and capillaries were readily identified (Fig. 2). One large embolus was associated with a simultaneous bone marrow embolus (Fig. 3). Additional sections were cut and stained by Weigert's, Holzer's, and Bodian's techniques in an attempt to demonstrate glial fibers and myelin sheaths. The Holzer and Bodian stains were faintly positive and the myelin sheath stain was negative. It was believed that this was due in part to the small size of the embolic fragments compared with the total amount of lung tissue.

### *Case 2*

R. H., a white male, 52 years old, was involved in an automobile accident and admitted to the hospital in coma. Roentgenograms showed a skull fracture and trephine was performed to relieve intracranial pressure. The patient died approximately 30 hours after admission. Necropsy (no. 4607) was performed 6½ hours post mortem

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and revealed a comminuted basal skull fracture with focal subdural, and diffuse subarachnoid, hemorrhage. The brain showed numerous lacerations externally and on section, with focal hemorrhages and softening in the pons and medulla. The lungs were hemorrhagic and edematous.

Microscopically the brain showed laceration and hemorrhage with loss of tissue. Sections of the lungs showed, in the pulmonary arterioles, multiple emboli of compact tissue in which glial cells and small blood vessels could be identified (Fig. 4). Many of the emboli were associated with extensive intra-alveolar hemorrhages and early necrosis of the parenchyma, typical of acute infarction of the lung. Special techniques to demonstrate glial fibers and myelin sheaths were inconclusive, only the Holzer stain giving a faintly positive reaction.

*Case 3*

Baby H., a white male 16 days old, was dead on admittance. Death had occurred about 1 hour previously with apparently suspicious circumstances. Necropsy (no. 3705) was performed 2 hours post mortem and revealed fractures of both parietal bones with separation of the occipital suture line, associated with subdural hemorrhage and tentorial tear. Section of the brain revealed intraventricular and subependymal hemorrhages. The lungs were of normal weight and showed only slight hyperemia.

Microscopically, sections of the brain were not available for review but had been reported as showing intracerebral hemorrhage. Sections of the lungs showed numerous minute emboli which tended to be aggregated in groups. These contained glial cells embedded in a compact stroma similar to that seen in the previous cases (Fig. 5). No neurons or arterioles were visible, and special stains gave negative results.

*Case 4*

C. C. was a white male, 19 years of age, who injured his head in a fall from a tree and was admitted to the hospital in coma. A cerebral decompression was performed but the patient died 12 hours after the accident and 7 hours postoperatively. Necropsy (no. 7848) was performed 5½ hours post mortem and revealed comminuted fractures of the vertex, left parietal bone, and base of skull. Extensive subarachnoid hemorrhage was present. The brain was swollen and showed lacerations of both cerebral hemispheres with focal hemorrhages in cerebrum and brain stem. The lungs were heavy and showed numerous, small, irregularly shaped areas of hemorrhage.

Microscopically, the brain showed hemorrhage and laceration. Sec-

tions of the lungs showed areas of intra-alveolar hemorrhage and occlusion of several arterioles by emboli made up of cells resembling glial cells in a compact stroma (Fig. 6). No neurons or vascular structures were identifiable in the emboli. Of the special stains, only Holzer's method yielded a faintly positive reaction.

## Discussion

Reported cases of emboli of brain tissue in the lungs are few. Krakower[3] reported a single microscopic embolus of brain tissue found incidentally in the lung of a 23-months-old child who died following a head injury. Oppenheimer[4] described a case of massive embolization of cortical tissue in a patient who underwent a decompression operation for skull fracture. At necropsy, emboli of brain tissue were visible grossly in the major pulmonary arteries; microscopically, the emboli contained well preserved nerve cells and glial and vascular elements, and were found in multiple sections of lung tissue. Ceelen[5] stated that embolic displacements of brain tissue are without doubt very rare and cited two cases from the German literature. In addition to traumatic embolization of brain fragments, several cases were reported in which ectopic glial tissue was found in the lungs in association with severe cerebral malformations. Askanazy,[6] in 1908, reported multiple foci of glial tissue in the lungs of a newly born child who also exhibited microcephaly and encephalocele. Hückel[7] reported a similar heterotopic growth in the lung of a 4-year-old child with frontal meningocele. Gruenwald[8] found glial tissue in the lungs of a 2-day-old child with partial anencephaly. Potter[9] reported 2 cases of ectopic brain tissue in the lungs of anencephalic monsters. All four authors considered the findings in the lungs to be the result of embolization of brain tissue by the blood stream. My 4 cases all showed multiple emboli of fragments of brain tissue with well preserved glial cells, occasional vascular structures, and, in one case, nerve cells.

In the period of July 1, 1937, to March 1, 1955, 213 complete necropsies have been performed in this hospital on patients with head injury. These cases are classified in Table I. The 4 cases showing emboli of cerebral tissue all fall into the first category, namely, that of skull fracture with severe laceration of the brain.

Of these 213 cases, 42 patients had undergone some form of surgery for decompression. In this connection it is of interest to note that in cases 2 and 4 the patients underwent intracranial surgery, as was true in the case described by Oppenheimer.[4] Furthermore, in cases 2 and 4 hemorrhagic areas in the lungs were found related anatomically to the vessels occluded by cerebral tissue. These may be interpreted as early

infarcts and their development is attributed to the length of survival of these patients after injury. In cases 1 and 3 the patients succumbed too rapidly for comparable changes to occur.

In addition to the 213 cases of head injury, a review was made of necropsies performed on patients dying after intracranial surgery for

TABLE I

*Classification of 213 Cases of Head Injury in Respect to Severity of Injury to the Brain*

| Type of injury | No. of cases | Percentage of total |
|---|---|---|
| Skull fracture with severe laceration of the brain | 89* | 41.8 |
| Skull fracture with less severe brain injury (e.g., contusion) | 56 | 26.3 |
| Brain laceration without skull fracture | 14 | 6.6 |
| Less severe brain injury without skull fracture | 16 | 7.5 |
| Skull fractures without significant brain injury | 9 | 4.2 |
| Gunshot wounds of head | 29 | 13.6 |
| Total | 213 | 100% |

* The 4 cases with pulmonary emboli of brain tissue were in this group.

various non-traumatic lesions. In 24 such cases during the 3-year period 1952–54, no evidence of embolization of cerebral tissue was seen. Oppenheimer's[4] case was the only one occurring in 277 complete necropsies on patients dying after intracranial surgery and in 42 cases of skull fracture in which the patients died without surgery. The present paper gives an incidence of 4 cases of emboli of brain tissue in 213 cases of severe head injury (approximately 2 per cent).

It would appear, therefore, that embolization of brain tissue to the lungs is by no means a rare phenomenon. The multiplicity of the emboli and the good preservation of glial cells were common to all reported cases. The inconclusive results obtained with special stains (Weigert's iron hematoxylin, Holzer's stain, Bodian's protargol stain) are attributed partly to the small size of the emboli and partly to postmortem changes in the emboli. The identification of neurons in one case and of vascular structures in another can be regarded as concrete evidence of the origin of the emboli in cases 1 and 2. The occurrence of cells resembling astrocytes in a compact stroma giving a positive glial reaction is believed to be strong presumptive evidence of the cerebral origin of the emboli in cases 3 and 4.

## SUMMARY

Four cases of embolization of cerebral tissue to the lungs are reported. All 4 cases were associated with skull fractures and laceration

of brain substance, and in one case simultaneous bone marrow embolization occurred. Glial cells were identifiable in all cases, vascular structures in 2 cases, and nerve cells were seen in one case. The multiplicity of the emboli and the preservation of their cellular elements are regarded as significant features in their identification. A review of necropsies of random cases of head injury gives an incidence of 2 per cent for this form of embolism.

## REFERENCES


1. Fisher, J. H. Bone-marrow embolism. *A. M. A. Arch. Path.*, 1951, **52**, 315–320.
2. Straus, R. Pulmonary embolism caused by liver tissue. *Arch. Path.*, 1942, **33**, 69–71.
3. Krakower, C. Pulmonary embolus containing cerebral tissue. *Arch. Path.*, 1936, **22**, 113–115.
4. Oppenheimer, E. H. Massive pulmonary embolization by cerebral cortical tissue. *Bull. Johns Hopkins Hosp.*, 1954, **94**, 86–89.
5. Ceelen, W. Die Kreislaufstörungen der Lunge. In: Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O. (eds.). J. Springer, Berlin, 1931, **3**, Pt. 3, 107–108.
6. Askanazy, M. Beiträge zu den Beziehungen zwischen Miss- und Geschwulstbildung anlässlich einer Beobachtung einer eigenartigen Schädelhernie mit Lungengliomen. *Arb. a. d. Geb. d. path. Anat. Inst. zu Tübingen*, 1908, **6**, 433–499.
7. Hückel, R. Über Gliaektopien in der Lunge bei angeborener vorderer Hirnhernie. *Verhandl. d. deutsch. path. Gesellsch.*, 1929, **24**, 272–279.
8. Gruenwald, P. Emboli of brain tissue in fetal lungs. *Am. J. Path.*, 1941, **17**, 879–884.
9. Potter, E. L., and Young, R. L. Heterotopic brain tissue in the lungs of two anencephalic monsters. *Arch. Path.*, 1942, **34**, 1009–1015.


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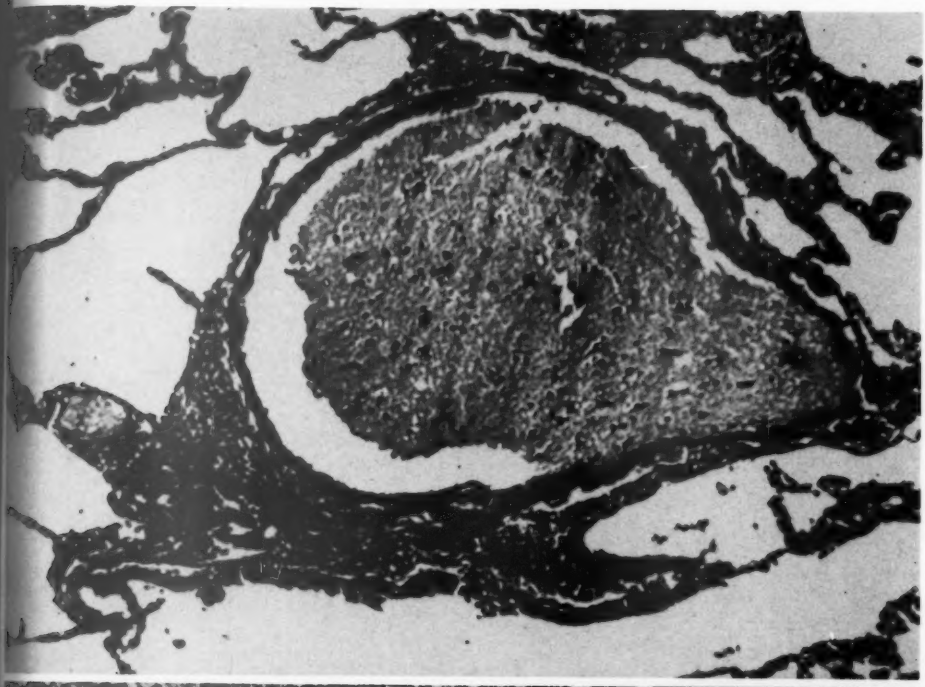
[ *Illustrations follow* ]

## LEGENDS FOR FIGURES

FIG. 1. Case 1. Section of lung showing occlusion of arteriole by an embolus of cerebral tissue. × 80.

FIG. 2. Case 1. Higher magnification of an embolus showing one neuron, one capillary, and numerous glial cells. × 445.



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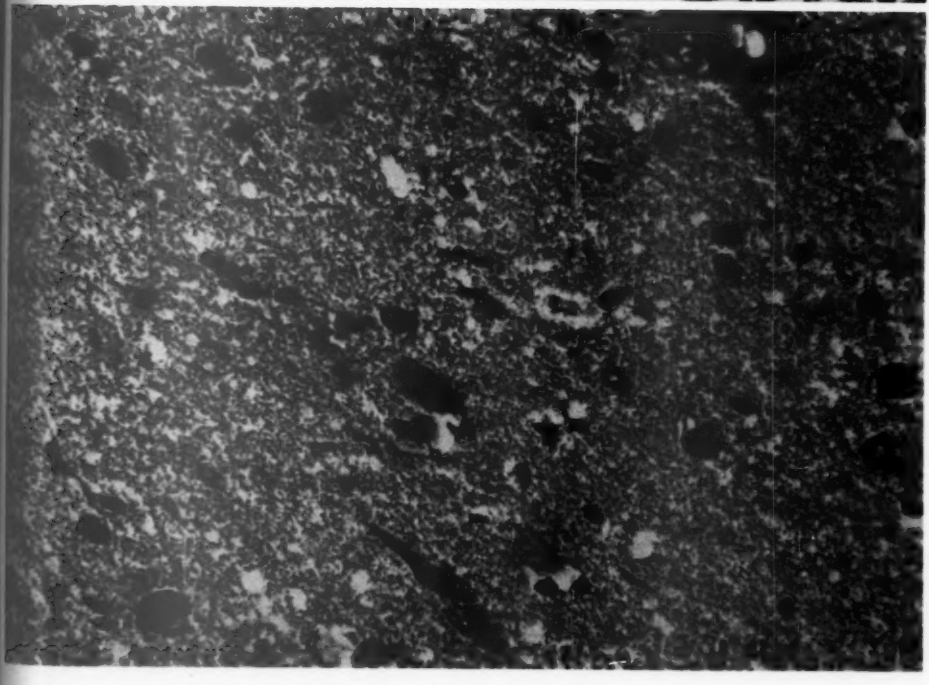

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FIG. 3. Case 1. Low-power view of part of a cerebral tissue embolus showing simultaneous bone marrow embolism. × 105.

FIG. 4. Case 2. High magnification of an embolus showing a cerebral capillary and a few poorly preserved glial cells in a compact stroma. × 620.

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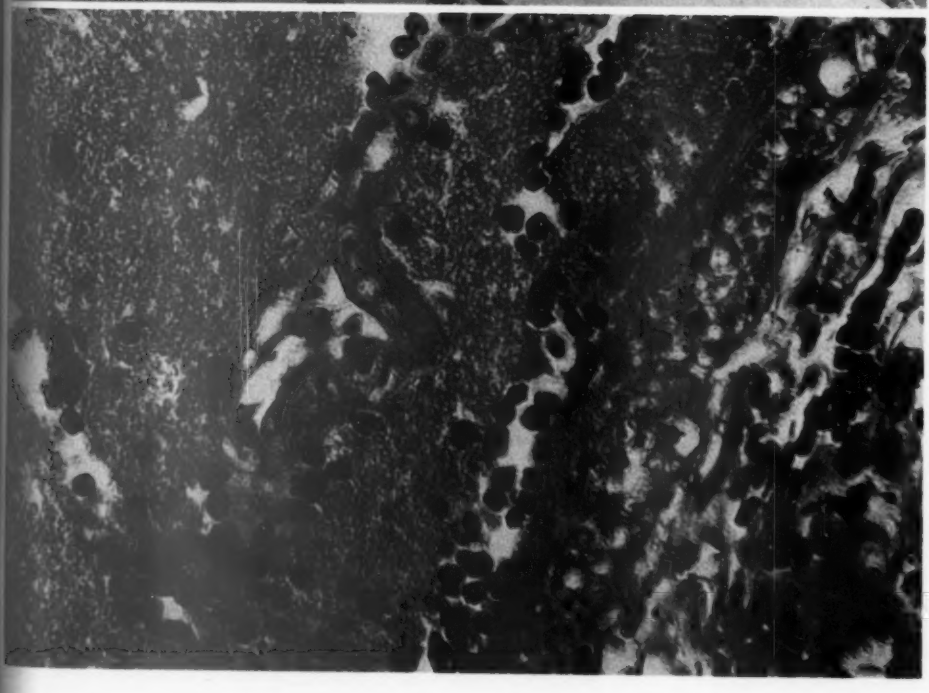

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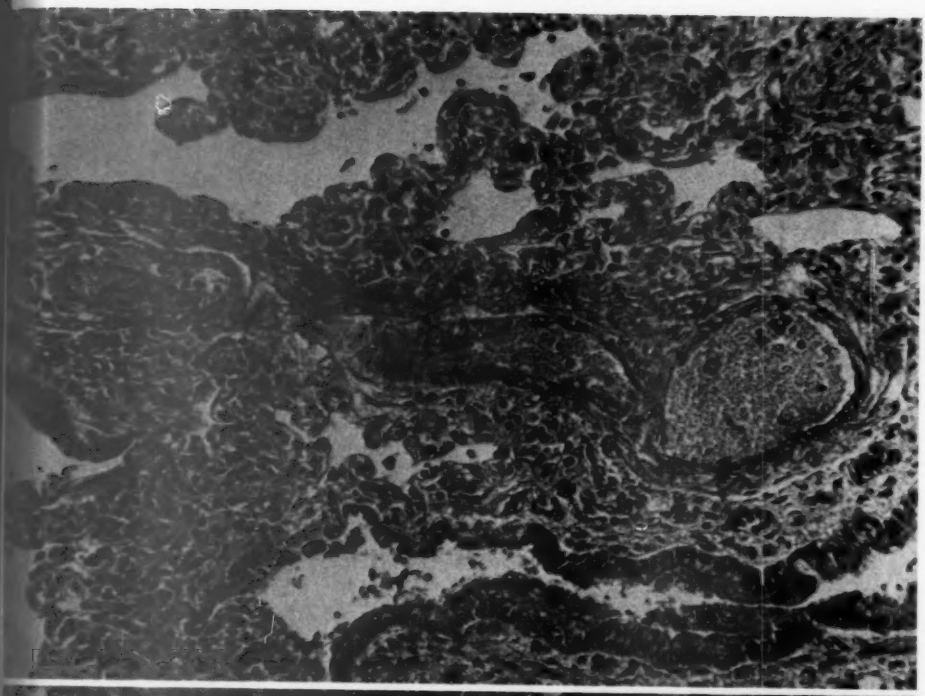
FIG. 5. Case 3. Embolus of glial tissue distending a pulmonary arteriole. × 220.

FIG. 6. Case 4. Small embolus of brain tissue in a pulmonary artery. × 265.



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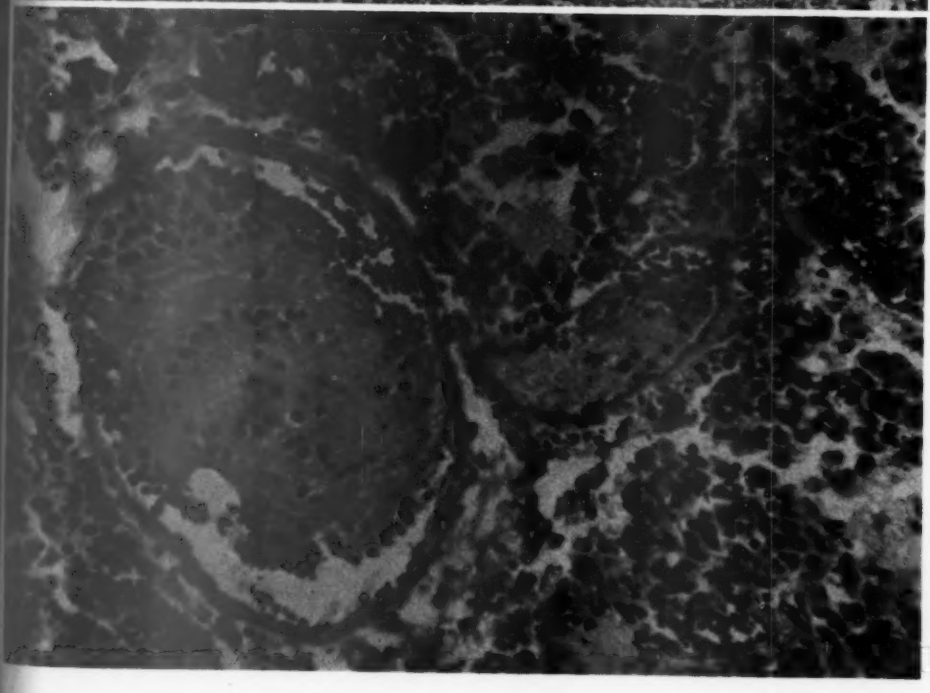

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# THE EFFECT OF HYPERCHOLESTEROLEMIA UPON INTIMAL REPAIR OF THE AORTA OF THE RABBIT FOLLOWING EXPERIMENTAL TRAUMA *

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Relatively little information is available concerning the onset and pattern of development of atherosclerosis. In a previous study from this laboratory,[1] it was pointed out that aortic intimal fibro-elastic plaques are a constant finding in all children over 2 weeks of age. Although lipid deposition was not a prominent feature of these early lesions, sudanophilic droplets were demonstrated both intracellularly and extracellularly within the deeper portions of these plaques. A morphologically identical lesion has been described by Moon and Rinehart[2] within the coronary arteries of young individuals who died suddenly without preceding debilitating illness. It seems reasonable to assume that these intimal alterations may be the earliest phase in the development of the adult atherosclerotic plaque. We have never observed changes within these intimal thickenings indicating that they are undergoing "spontaneous regression."

The cellular composition of these plaques in children suggested to us that they might represent reparative activity following injury to the intima. This concept was of additional interest since most authorities concede that there is an element of local injury or damage within the intima associated with lipid precipitation as an initial stage in the pathogenesis of the human plaque. Pertinent also to this hypothesis is the recent re-emphasis of the concept that atherosclerotic plaques may be mural thrombi which have undergone organization and degenerative phenomena.[3-7] Accordingly, Prior and Hutter[8] traumatized the aortic intima of rabbits to study vascular injury and repair, and to contrast the changes observed in these mural thrombi with the fibro-elastic plaques of infants. A lesion grossly resembling an early plaque in the human was noted 35 days after intimal injury in the experimental animal; and this lesion was histologically identical with the fibro-elastic thickenings of children. Although lipid material and macrophages were never a prominent feature of this experimentally produced lesion, calcification was seen as early at 49 days after injury

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although its location and appearance differed from that observed in the human atherosclerotic lesion.

The present investigation was an attempt to study the effect of hypercholesterolemia upon segments of the previously traumatized rabbit aorta. Although there are points of similarity between human and cholesterol arteriosclerosis, Duff and McMillan[9] have pointed out that great caution should be exercised in transposing interpretations derived from studies of experimental cholesterol atherosclerosis into terms supposedly applicable to the human disease. The present experiment was designed with the hope that the combination of intimal trauma and hypercholesterolemia would produce lesions similar to the human process and add some knowledge of the pathogenesis of the atherosclerotic plaque.

## Methods

Twenty-seven New Zealand white rabbits, approximately evenly divided as to sex, were used in this study. Their age at the beginning of the experiment was 8 to 10 weeks and their average weight was 2,200 gm. They were housed separately in wire cages and fed Purina rabbit chow supplemented with 1 gm. of cholesterol per day. The cholesterol was administered as the purified substance dissolved in ether, mixed with the food pellets, and allowed to evaporate. Blood cholesterol values at the beginning of the study ranged between 20 and 110 mg. per 100 cc. The cholesterol supplement was instituted on the same day that intimal trauma was carried out.

The animals were anesthetized with pentothal and ether, and the peritoneal cavity was incised under sterile precautions. The abdominal viscera were retracted from a segment of lumbar aorta and the latter was freed from the adjacent connective tissue with a minimum of trauma. The aorta was then pierced at an oblique angle with a 24-gauge needle, the point of which was deliberately angulated to provide a barbed instrument. The aortic intimal surface was then traumatized by repeated vertical and horizontal motions. The needle was withdrawn and hemostasis affected by means of direct pressure over the puncture site. Before closing the peritoneal cavity the traumatized area was marked by silk sutures placed within the peri-aortic connective tissue.

The animals were sacrificed at intervals ranging from 2 days to 8½ months following intimal injury. As will be pointed out later, it was necessary to sacrifice the animals at approximately 1 week intervals during the first 3 months of the experiment. The entire aorta was removed and areas at a distance from the traumatized segment were considered to serve as controls for cholesterol effect only. Blood cholesterol determinations were obtained on all animals at the time of

sacrifice. These ranged between 600 and 800 mg. per 100 cc., levels of 600 mg. being present as early as 14 days after commencement of the cholesterol feeding.

## Pathologic Findings

The technique did not permit control of the depth of the trauma, so that the tissue injury varied from an intimal defect only to foci in which nearly the entire thickness of the vessel wall had been traumatized. In general, we were particularly interested in those areas in which the trauma had been more superficial, i.e., had involved the intima and inner media only.

In gross appearance, the injured intimal surface 2 days following trauma presented a pitted area with raised, swollen margins. Microscopically, wide disruption of the intimal and medial coats was present. The medial cellular elements in the involved areas showed nuclear variation and shrinkage and there was evidence of edema and fibrin deposition. A moderate number of polymorphonuclear leukocytes were noted within the adventitia. Fibroblastic activity was prominent at the margins of the lacerated intima and also in the adventitial coat. No new capillaries could be identified although there was some attempted endothelial bridging of the denuded intima. Frequently the lacerated vessel lining protruded into the lumen as a polypoid mass (Fig. 1). At 7 days the core of these polypoid areas still consisted of fibrin and cellular débris, although the surface was now completely endothelized (Fig. 2). Where depressions had been present in the vessel lining, the gaps were replaced by active fibroblastic tissue growing perpendicular to the long axis of the vessel and covered by a new endothelial lining. Broad zones of hyaline material persisted within the damaged media and tended to be surrounded by proliferating fibroblasts. A few new capillaries extended from the vasa vasorum into the inner media.

Lipophages were first observed 14 days following the initial injury (Fig. 3). They were concentrated about the junction of the intact media and newly formed fibrous cap. This cap was very cellular and the fibroblasts were oriented in a plane parallel to the long axis of the vessel. Weigert-van Gieson's staining showed that the intercellular material was composed of large amounts of elastic tissue and scant collagenous fibers (Fig. 4). In areas where the trauma had involved the adventitia, calcification of the necrotic débris was noted. The vasa vasorum were prominent and new endothelium-lined channels could be traced lumenward from these vessels. While the source of the lipophages cannot be stated categorically, it is our impression that they were altered wandering cells which apparently entered through the vasa vasorum, since their migration from the vessel lumen through

the dense avascular fibro-elastic cap is extremely unlikely. It is impossible to deny, however, that some may arise *de novo* through transformation from fibroblasts.

The location of the lipophages in the traumatized segment at this stage is in marked contrast to their position in the earliest spontaneous (hypercholesterolemia) or non-traumatized lesions noted in these animals. The earliest microscopic spontaneous lesions were seen at 27 days and consisted of a tightly packed row of lipophages located between the internal elastic membrane and the endothelium (Fig. 5). We were impressed by the similarity between the experimentally produced lesion at 14 days and moderately advanced human lesions observed in coronary arteries and aorta. This similarity is readily apparent by referring to MacCallum's[10] Figure 185 which is an illustration of a human coronary artery encroached upon by a thick, atherosclerotic plaque. Common to both are raised hillocks composed of dense fibro-elastic tissue, some destruction of the musculo-elastic layer, and collections of large lipophages in the basilar portion of the plaque.

At 27 days after injury, when the first spontaneous lesions which have been referred to, were appearing, the traumatic lesions were showing some hyaline change within the fibrous tissue cap and occasional plaques disclosed beginning necrosis within the subendothelial zone. The spontaneous lesions showed fibrosis and some necrosis at 36 days.

Forty-six days after trauma the aorta showed grossly such a number of lesions of the spontaneous type that it was sometimes difficult to identify the traumatized segment. The spontaneous plaques were oval and elongated in the long axis of the aorta. They were definitely raised above the intimal surface, light yellow, and showed some tendency for fusion of adjacent plaques (Fig. 6). The traumatized areas presented as transversely oriented, plateau-shaped elevations which had a darker yellow color than the spontaneous type (Fig. 7). Small polypoid excrescences frequently could be identified along the margin of the transverse ridges. There were, however, striking similarities between the microscopic appearance of lesions of the spontaneous and of the traumatic type at 46 days. The spontaneous variety appeared as a conchoidal intimal thickening which was very rich in lipophages (Fig. 8). The latter were most concentrated along the internal elastic membrane although they were generally diffuse. A few foci of pyknosis and karyorrhexis were present, but this was not impressive. The Weigert-van Gieson stain showed the intercellular material to be devoid of elastic or collagenous elements. The internal elastic membrane was intact and no evidence of medial disease was

noted. The traumatically induced lesions were larger, flatter, and had a much thicker intima than the spontaneous variety (Fig. 9). The lipophages were fewer and were also concentrated in the basal portion of the plaque. Necrosis was prominent throughout the plaque and extended medialward so that no well defined internal elastic membrane could be identified.

This process continued until at 97 days after injury it was no longer possible to separate the advanced spontaneous lesions from those traumatically induced (Figs. 10 and 11). Both were composed chiefly of acellular necrotic débris in which some lipophages could still be identified. We were not impressed with either reparative connective tissue or significant dystropic calcification within the spontaneous or traumatic lesions over long periods, i.e., up to 8½ months after trauma. The animals which were maintained on oral cholesterol over this long period tended to show minimal calcification of the necrotic fatty material which was more noticeable in lesions of the traumatic type.

## Discussion

The sequence of events following cholesterol feeding in the rabbit and resulting in the experimental atherosclerotic plaque has been studied carefully.[11,12] In young rabbits fed 1 gm. of cholesterol daily in Purina rabbit chow, the earliest lesions were foam cells and intercellular lipids located between the internal elastic membranes and the endothelium. These early lesions were arranged parallel with the vessel axis but increased in width until they extended completely around the vessel lumen. Between the foam-laden cells were fine strands of fibroglia, elastic fibrils, and conspicuous ground substance. The foam cells in the deep portion became necrotic and left the fatty material free in the finely granular débris. Fibroblasts proliferated extensively in the deeper layers of the intimal thickening and there was an increase in fibroglia and elastic fibers. The pool of free fatty substance became calcified and calcification might extend to involve the underlying media.

Duff[11] described focal necrosis of muscle fibers in the media before any intimal changes were observed. Medial damage of the more severe degrees always had associated a more or less marked change in the overlying intima. With progression of the medial lesions there occurred destruction of muscle and elastic tissue and an increasing infiltration of lipids. The internal elastic membrane eventually underwent complete disintegration and the inner layer of the media, packed with fat-laden foam cells, became almost indistinguishable from the intima.

While most of our observations have been concentrated upon rab-

bits fed cholesterol over a period of 3 months, fibroblastic activity seemed minimal in the spontaneous lesions throughout this period. There was, however, active fibroblastic proliferation in the traumatized segments reaching a maximum at 14 days. This lesion, which so closely resembled the human atherosclerotic plaque, showed prominent necrosis 27 days after injury. From this time on the traumatic lesion was characterized by progressively increasing necrosis, until 46 days after injury it was difficult to separate the traumatic from the spontaneous lesions on a histologic basis. Traumatic lesions alone, however, in animals maintained on a cholesterol-free diet, showed no inhibition of connective tissue repair and a dense fibro-elastic plaque was produced in 35 days after injury.[8] Degenerative changes, other than rare calcific deposition, were unusual in these intimal thickenings and lipid deposition was never a prominent feature, minute amounts being occasionally seen extra-cellularly.

A review of the mechanically induced vascular traumatic lesion has been presented in a previous publication.[8] Pertinent to the present investigation is a brief mention of the results of traumatic procedures carried out in the experimental animal maintained on a high cholesterol diet. The present study has confirmed the observation that traumatized areas accumulate lipid at a much faster rate than those not traumatized, the earliest spontaneous lesions being noted at 27 days while those of the traumatic variety were rich in foam cells 14 days after the injury.

Ssolowjew traumatized the rabbit aorta by adventitial cauterization[13] and also studied changes in the carotid artery which had been subjected to an exteriorization procedure.[14] It is of interest that in the former experiment abundant lipid deposition in the injured media was observed after 14 days, when cauterization was performed coincidentally with the commencement of cholesterol feeding. Extracellular and intracellular fat deposition was noted in the damaged media of the exteriorized vessel while the adjacent normal vessel was free of fat. Displacement of the carotid artery into a skin bridge produced transverse lacerations of the elastic lamellae. Where tears of the elastic tissue occurred, there was increased permeability to substances present in the blood (trypan blue). After some time, however, the local increased permeability was counteracted by reparative processes. The lipid substances circulating in the blood after induction of lipidemia invaded the arterial wall exactly like the stain and accumulated wherever its permeability had been increased (where the elastica was torn). During the later stages of the reparative process (after intimal thickening had developed), no further lipids were deposited in sites originally occupied by tears in the elastic lamellae.

Kelly *et al.*[15] studied the combination of induced intimal proliferation secondary to medial degeneration and localized lipid deposition from prolonged hypercholesterolemia in both old and young rabbits. The daily cholesterol intake in these animals was 0.06 gm. per lb. of body weight. After 22 weeks on the diet, localized lesions in the abdominal aorta and renal artery were produced by the freezing procedure of Taylor *et al.*[16] Animals in the hypercholesterolemic series were sacrificed at intervals of 2 to 52 weeks after freezing. The frozen areas underwent the same sequence of medial degeneration and fibroblastic intimal regeneration as described by Taylor in animals not fed cholesterol. The lesions were predisposed to lipid accumulation and with this there was interference with intimal regeneration. The localization of lipids was conspicuous in the degenerated media of the renal artery but not in the aorta. The importance of an intact internal elastic membrane in preventing lipid infiltration of the degenerated media was apparent.

Harrison's[17] experimental arterial disease produced by cholesterol and vitamin D is pertinent to this discussion. He carried out two experimental procedures: in the first, cholesterol sclerosis was induced in the rabbit aorta as a preliminary lesion and vitamin D sclerosis was later superimposed; in the second, vitamin D sclerosis constituted the preliminary lesion and cholesterol was then added. In both cases the second lesion was confined to the parts of the vessel left unaffected by the first lesion. Both cholesterol sclerosis and vitamin D sclerosis render the affected parts of the vessel wall relatively immobile and this immobility appears to make them less susceptible to the subsequent lesion. He concluded that the movements of the vessel determine the localization of both vitamin D and cholesterol lesions, and probably the human disease.

## Summary

The reaction to a form of mechanically induced trauma to the intimal surface of the rabbit aorta in the presence of hypercholesterolemia has been described. The injured segment showed increased permeability to cholesterol and 14 days after trauma a plaque composed of lipid material and dense connective tissue was produced. This lesion was histologically identical with early human atherosclerotic plaques as seen in the coronary arteries and aorta. This experimentally produced lesion subsequently was characterized by necrosis and inhibition of connective tissue so that 46 days after injury the spontaneous and traumatically induced plaques were structurally similar. This inhibitory effect of cholesterol upon connective tissue repair may in part explain why the histologic counterpart of the advanced human atherosclerotic lesion cannot be duplicated experimentally by cho-

lesterol administration. A brief review of the results of other combinations of vascular trauma and hypercholesterolemia has been presented.

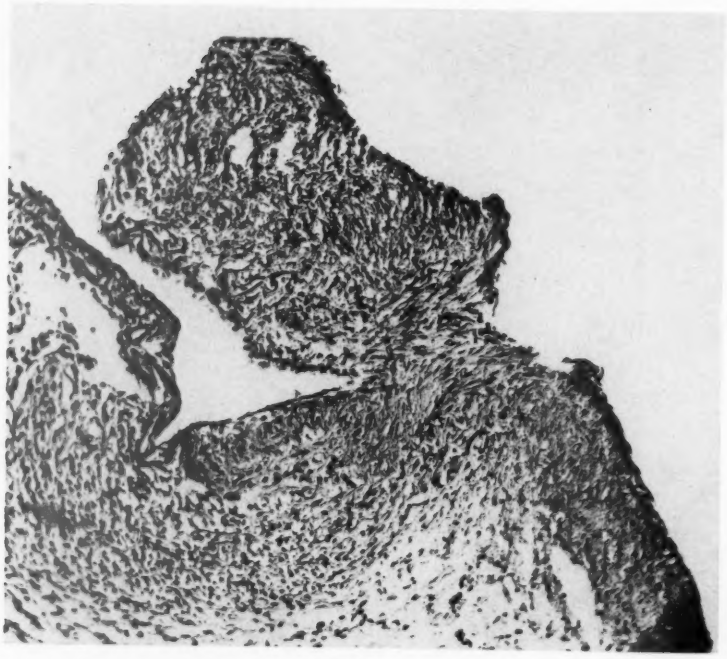
## REFERENCES


1. Prior, J. T., and Jones, D. B. Structural alterations of the aortic intima in infancy and childhood. *Am. J. Path.*, 1952, **28**, 937–951.
2. Moon, H. D., and Rinehart, J. F. Histogenesis of coronary arteriosclerosis. *Circulation*, 1952, **6**, 481–488.
3. Duguid, J. B. Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. *J. Path. & Bact.*, 1946, **58**, 207–212.
4. Duguid, J. B. Thrombosis as a factor in the pathogenesis of aortic atherosclerosis. *J. Path. & Bact.*, 1948, **60**, 57–61.
5. Duguid, J. B. Pathogenesis of atherosclerosis. *Lancet*, 1949, **2**, 925–927.
6. Duguid, J. B. The arterial lining. *Lancet*, 1952, **2**, 207–208.
7. Crawford, T., and Levene, C. I. The incorporation of fibrin in the aortic intima. *J. Path. & Bact.*, 1952, **64**, 523–528.
8. Prior, J. T., and Hutter, R. V. P. Intimal repair of the aorta of the rabbit following experimental trauma. *Am. J. Path.*, 1955, **31**, 107–123.
9. Duff, G. L., and McMillan, G. C. Pathology of atherosclerosis. *Am. J. Med.*, 1951, **11**, 92–108.
10. MacCallum, W. G. A Text-book of Pathology. W. B. Saunders Co., Philadelphia, 1936, ed. 6, p. 327.
11. Duff, G. L. Experimental cholesterol arteriosclerosis and its relationship to human arteriosclerosis. *Arch. Path.*, 1935, **20**, 81–123.
12. Kuntz, A., and Sulkin, N. M. Lesions induced in rabbits by cholesterol feeding, with special reference to their origin. *Arch. Path.*, 1949, **47**, 248–260.
13. Ssolowjew, A. Experimentelle Untersuchungen über die Bedeutung von lokaler Schädigung für die Lipoidablagerung in die Arterienwand. *Ztschr. f. d. ges. exper. Med.*, 1929–30, **69**, 94–104.
14. Ssolowjew, A. Über experimentell hervorgerufene Elasticarisse der Arterien und deren Bedeutung für die Lipoidablagerung. *Virchows Arch. f. path. Anat.*, 1932, **283**, 213–230.
15. Kelly, F. B., Jr.; Taylor, C. B.; Haas, G. M., and Craig, J. M. Arteriosclerotic lesions in areas of vascular injury in hypercholesterolemic rabbits. *Circulation*, 1951, **4**, 463.
16. Taylor, C. B.; Baldwin, D., and Haas, G. M. Localized arteriosclerotic lesions induced in the aorta of the juvenile rabbit by freezing. *Arch. Path.*, 1950, **49**, 623–640.
17. Harrison, C. V. Experimental arterial disease produced by cholesterol and vitamin D. *J. Path. & Bact.*, 1933, **36**, 447–453.


## LEGENDS FOR FIGURES

FIG. 1. Traumatized segment of a rabbit's aorta after 2 days. The core of the polypoid mass is composed of avulsed, degenerating media and intima. × 138.

FIG. 2. Traumatized segment at 7 days showing endothelization of the polypoid mass and fibroblastic replacement of a V-shaped defect above it. × 138.



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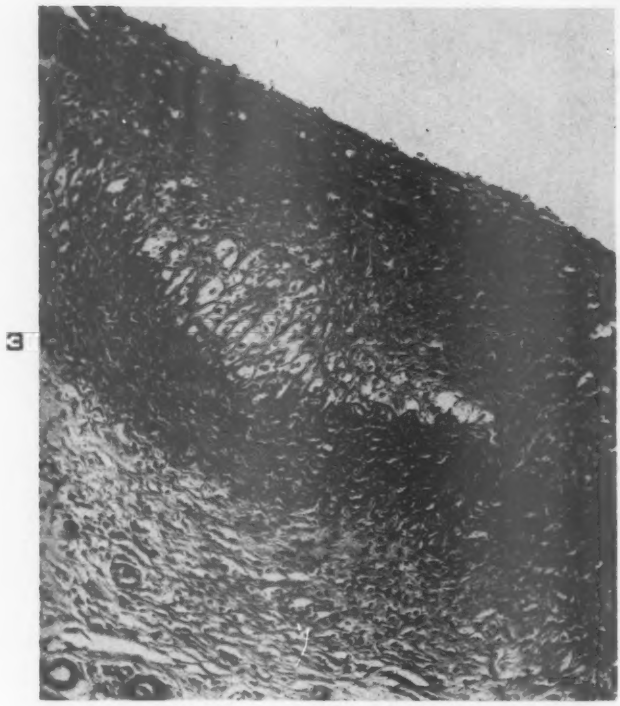
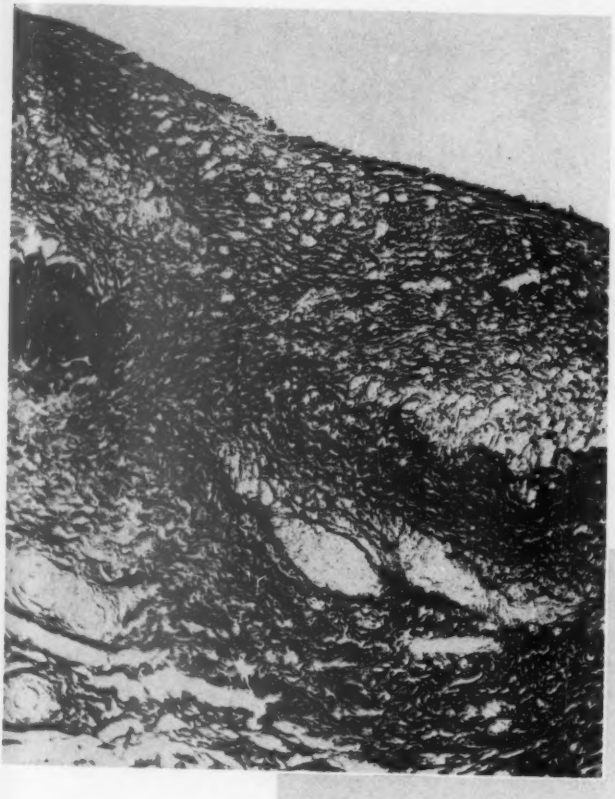


FIG. 3. Traumatized segment 14 days after injury. The collection of lipophages in the deeper tissue covered by a thick fibro-elastic cap presents a striking similarity to the early human plaque. × 138.

FIG. 4. Weigert-van Gieson's stain from the same block as the section shown in Figure 3. The intimal thickening is rich in elastic tissue, with scant collagen being present. × 138.

FIG. 5. Non-traumatized segment of rabbit aorta 27 days after institution of cholesterol feeding. × 227.



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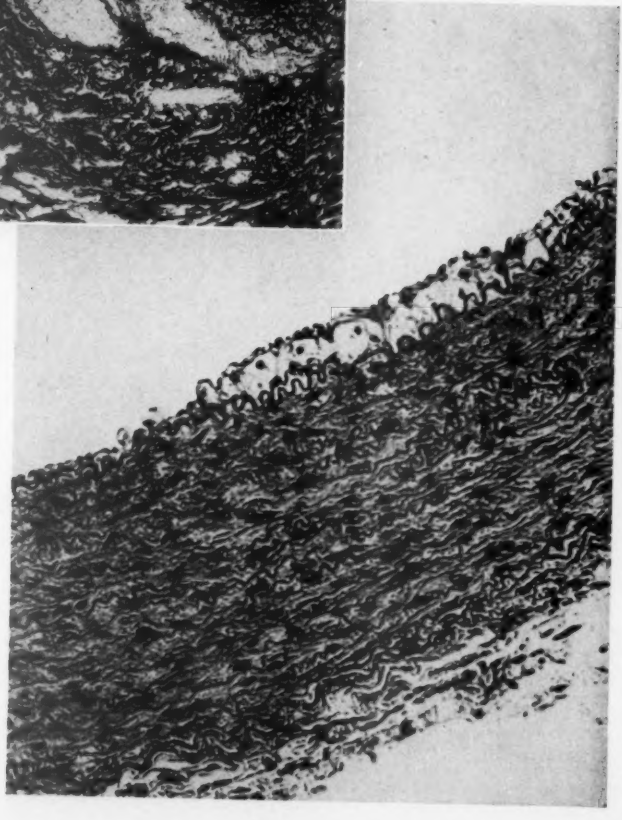

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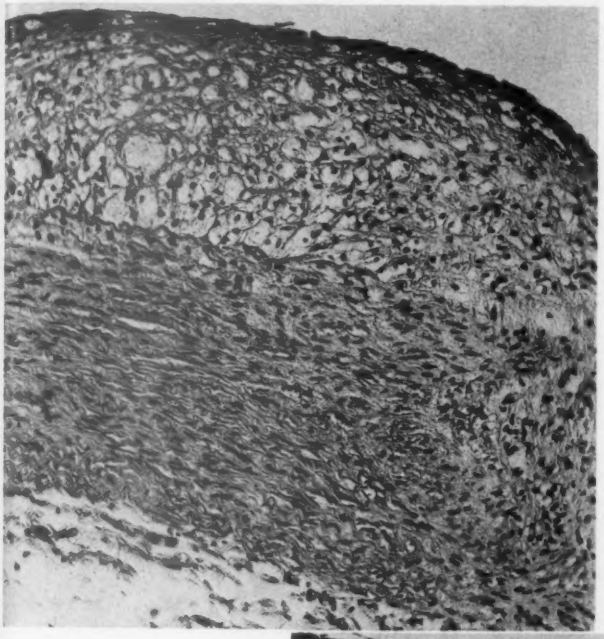
FIG. 6. Spontaneous lesions (non-traumatized) at 46 days. The oval-shaped plaques are elongated in the long axis of the aorta. × 3.5.

FIG. 7. Traumatized segment at 46 days. The transverse plateau-shaped elevations show small polypoid nodules along one margin. × 7.

FIG. 8. Spontaneous lesion at 46 days. The lesion is rich in lipophages and shows a moderate degenerative change. × 160.

FIG. 9. Traumatic lesion at 46 days showing extensive necrosis, some lipophages, and an ill defined internal elastic membrane. × 160.



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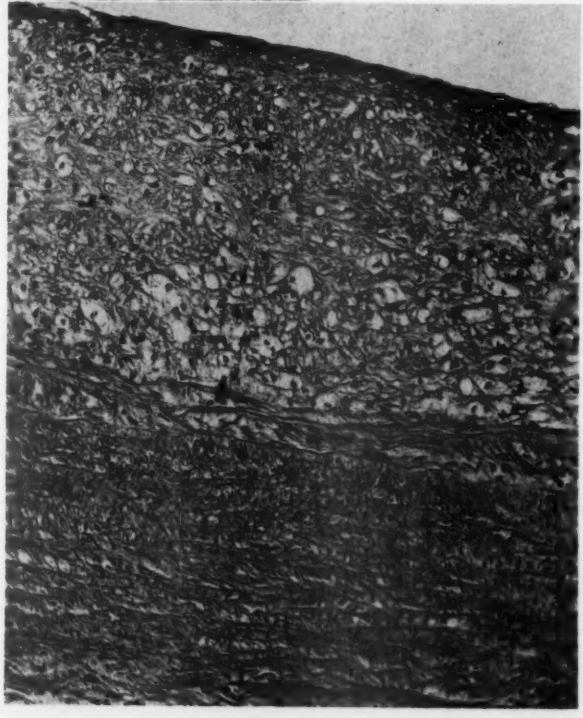

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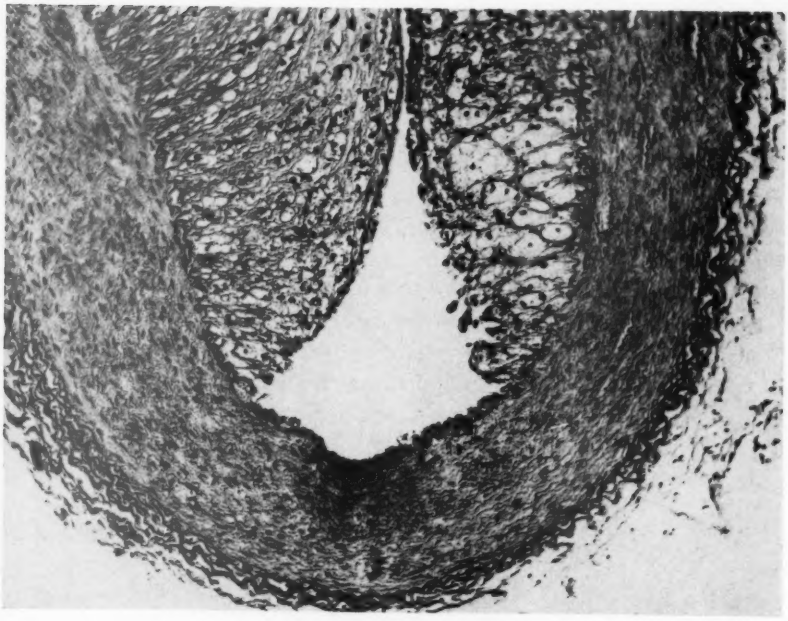
FIG. 10. Spontaneous lesions at 97 days. The younger lesion occupies the right half of the lumen. × 141.

FIG. 11. Traumatic lesion at 97 days composed of acellular necrotic débris and some lipophages. × 179.



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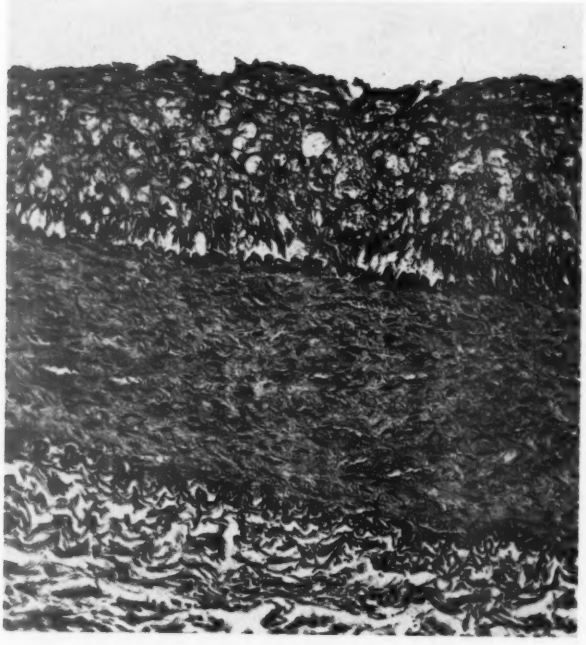

11

# THE PATHOGENESIS OF RETICULO-ENDOTHELIAL FOAM CELLS

## Effect of Polyvinylpyrrolidone on the Liver of the Mouse *

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In the search for an efficient, readily available, and inexpensive plasma expander, many substances have been investigated. Relatively few have turned out to be acceptable. Polyvinylpyrrolidone (PVP), a synthetic polymer, has been shown to meet most of the requirements satisfactorily. However, the fate of the portion which is not excreted during the first few days has not been determined completely.

When PVP is administered to mice intravenously, intraperitoneally, or subcutaneously, some of the colloidal particles are taken up by cells of the reticulo-endothelial system, including the macrophages of the liver. This results in the development of foam cells which is interpreted as presumptive evidence of PVP storage. Foam cell development in reticulo-endothelial tissues has resulted from the administration of many macromolecular substances,[1-3] and has been accepted as a criterion for estimating the degree of histologic change in the liver resulting from PVP administration.[4,5]

The results of early investigations on PVP storage show much disagreement and a survey of the literature reveals that this disagreement is due, to a considerable degree, to variations in experimental procedures, particularly in reference to quantity of PVP administered, the time elapsed between administrations of PVP and removal of tissues, the species of animals used, and the range of molecular weight of the PVP used. This investigation attempts to correlate the histologic changes brought about in the liver of the mouse with these variables.

## Materials and Methods

The animals used were albino mice of both sexes from the inbred strains, BUA, BUB, and BUC maintained in the biological laboratory of Brown University. The mice were between 60 and 90 days of age when the experiments were begun, and they were maintained on a diet of Purina laboratory chow.

Mice were killed by a sharp blow on the head followed by decapitation. After the animals were bled as thoroughly as possible, the large

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left lateral lobe of the liver was removed quickly and small blocks of liver were taken for histologic preparation. As a routine, tissues were fixed 24 hours in Bouin's fluid, embedded in paraffin by the dioxane technique, sectioned at 5 $\mu$, and stained in Delafield's hematoxylin and eosin.

*PVP Solutions*

Several preparations of PVP were employed in these experiments.

(1) PVP (Macrose) was supplied by the Schenley Laboratories. It was contained in infusion bottles as a sterile solution, free of foreign matter, containing 3.5 per cent by weight of PVP, plus inorganic salts. This material is reported to have an average molecular weight of about 40,000, with a range of 10,000 to 80,000; and its viscosity coefficient is designated as K-30.

(2) PVP of the same K-value as that described under (1) was supplied in powder form by the Schenley Laboratories (Lot 10105-1-101). This material was used to make 10 and 20 per cent solutions of PVP in 0.9 per cent NaCl.

(3) A preparation of lower molecular weight designated as K-20 also was supplied by the Schenley Laboratories in powder form (Lot 885789-1). This material is reported to have an average molecular weight of approximately 20,000. From this preparation, 5, 10, and 20 per cent solutions of PVP in 0.9 per cent NaCl were prepared.

(4) Dr. W. O. Ney of Arnold, Hoffman and Co., Providence, R.I., provided a preparation of high molecular weight in the form of a 20 per cent solution made up in distilled water and having a K-value of approximately 71 (Lot DS-107). The mean molecular weight of this highly polymerized material is about 125,000. To facilitate injection, this viscous 20 per cent solution was diluted to 5 per cent with distilled water.

*Foam Cell Counts*

During the course of this work, the need for a reliable method of estimating quantitatively the extent of alteration in liver tissue after PVP administration became evident. Subjective evaluation was abandoned in favor of counting foam cells. Bouin-fixed preparations, stained in hematoxylin and eosin, were used for these counts. With a 7.5 $\times$ ocular and 45 $\times$ objective, the sinusoidal lining cells in a field outlined by 16 squares of a Howard micrometer disk were counted. At the same time, the lining cells which appeared as foam cells were counted. This was repeated in three widely scattered fields of each section, and each field was selected to include all zones of a lobule from the central vein to the portal area. Before counts were made, each field was examined under low magnification to be sure that it

appeared to be representative of the whole section. For comparison, the counts were expressed as percentage of foam cells among all sinusoidal lining cells.

In order to determine whether a series of counts made on a single section would show good correspondence, ten counts were made in different areas of a section from experiment 65. The mean percentage of foam cells for the ten areas was 21.7 ± 6.46 (S. D.). The mean deviation of the ten counts was 4.79. It is apparent that the counts showed fairly good correspondence and that the counting procedure is sufficiently accurate to approximate the percentage of foam cells in sections of liver tissue.

### *Program of Injection and Sacrifice of Animals*

For each injection the mice usually received 0.5 ml. of PVP solution, which is approximately equivalent to 20 to 25 per cent of the total blood volume of the young adult mice used in these experiments. The total number of injections, as well as the route of injection, varied depending upon the experiment. When mice were given a series, the injections usually were administered daily, on alternate days, or every third day. Table I presents the plan of experiments in which low molecular weight PVP (K-20) was employed. Similar plans for medium (K-30) and high (K-71) molecular weight PVP are presented in Tables II and III.

## Experiments and Observations

### *General Histologic Observations*

When slight to moderate alterations were produced in the liver of the mouse, some Kupffer cells appeared swollen and projected into the lumina of the sinusoids (Fig. 1). In this swollen state the cytoplasm was finely granular. The nuclei were condensed and hyperchromatic. The distended Kupffer cells contained vacuoles of various sizes which gave them the appearance of typical foam cells (Fig. 1). Scattered foam cells appeared in the periportal connective tissue. Leukocytic aggregations were observed occasionally, particularly in the area surrounding the portal veins and bile ducts. The relationship of leukocytic infiltration to PVP has not been clarified, but such infiltrations were more conspicuous in the injected mice than in normal ones.

When more extensive histologic alterations were produced in the liver, the degree of vacuolization of the cytoplasm of macrophages was greater. The vacuoles always appeared optically empty, although they were surrounded by finely granular cytoplasm. The foam cells were more numerous, and frequently were seen in aggregates which formed

nodules (Figs. 1, 2, and 3). These nodules were located midzonally in the lobule (Figs. 1 and 3) as well as in the region of the portal triad (Fig. 2). More extensive infiltrations of leukocytes occasionally were found in the periportal area, particularly around bile ducts (Fig. 4). Nodules of foam cells, wherever located, sometimes were invaded by leukocytes.

Except for the usual diurnal variation in the appearance of the parenchymal cells, especially in reference to glycogen storage,[6] changes were not encountered in the parenchyma of the liver. PVP was not demonstrated in parenchymal cells.

### *Results of Varying the Average Molecular Weight of the PVP Preparations*

*Low Molecular Weight.* Table I presents the results of foam cell counts performed in experiments with the K-20 preparation of low molecular weight.

A single 0.5 ml. injection of a 20 per cent solution containing 100 mg. of this material brought about no change in the histologic picture of the liver after 30 days (experiment 20).

TABLE I

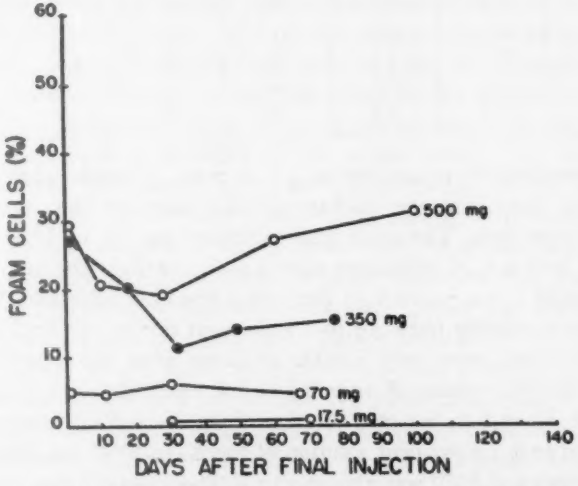
*Foam Cell Counts in Experiments Employing PVP (K-20) of Low Molecular Weight*

| Experiment number | Total volume | Concentration | Quantity of PVP | Days after first injection | Days after final injection | Percentage of foam cells |
|---|---|---|---|---|---|---|
| | *ml.* | % | *mg.* | | | |
| 20 | 0.5 | 20 | 100 | 30 | 30 | 0.0 |
| 131 | 2.0 | 10 | 200 | 7 | 1 | 2.8 |
| 132 | 2.0 | 10 | 200 | 17 | 11 | 10.1 |
| 133 | 2.0 | 10 | 200 | 36 | 30 | 7.2 |
| 134 | 2.0 | 10 | 200 | 73 | 67 | 4.6 |
| 21 | 4.0 | 20 | 800 | 15 | 3 | 19.3 |
| 24 | 4.0 | 20 | 800 | 49 | 37 | 16.2 |
| 26 | 4.0 | 20 | 800 | 78 | 66 | 8.4 |
| 27 | 4.0 | 20 | 800 | 102 | 90 | 9.9 |

A group of mice was injected with 0.5 ml. of a 10 per cent solution of this material on alternate days until four injections had been given. These animals received a total of 2.0 ml. containing 200 mg. of PVP. The mice were sacrificed at 1, 11, 30, and 67 days after the final injection (experiments 131 to 134 in Table I). With this quantity of PVP, slight alteration took place in some of the sinusoidal lining cells, resulting in foam cell counts ranging from 2.8 to 10.1 per cent. The explanation for the fairly high count of 10.1 per cent at 11 days (experiment 132) is not apparent.

In experiments 21, 24, 26, and 27 (Table I) the mice received a large quantity of the PVP with a low average molecular weight. They were injected with 0.5 ml. of a 20 per cent solution eight times over a 12-day period, the total volume of 4.0 ml. containing 800 mg. of PVP. Animals of this group were sacrificed at 3, 37, 66, and 90 days after the final injection, at which times the foam cell counts were 19.3, 16.2, 8.4, and 9.9 per cent, respectively. The foam cell formation in this series of experiments was moderate, with the higher percentages appearing at the shorter intervals. At 90 days, however, 9.9 per cent of the lining cells still could be classified as foam cells. A greater percentage of foam cells of the highly vacuolated type was observed than in the series of experiments in which the animals received only 2.0 ml. of the same PVP solution (experiments 131 to 134). Leukocytic infiltration was quite extensive but occurred predominantly as periportal aggregations and rarely in the scattered nodules located elsewhere.

*Medium Molecular Weight.* Table II presents the data for experiments with the K-30 PVP preparation of medium molecular weight. The results of these experiments are shown graphically in Text-figure 1.




Text-figure 1. Percentage of foam cells in the livers of mice which received different doses of PVP (K-30) of medium molecular weight.

In experiments 101 and 102, mice were given single injections of this material and sacrificed at 30 and 70 days after injection. Each mouse received 0.5 ml. of a 3.5 per cent solution containing 17.5 mg. of PVP. The percentage of foam cells after each interval was less than 1 per cent.

In the series consisting of experiments 11, 13, 14, and 15 (Table II), as well as in the previous series (experiments 101 and 102), the 3.5 per cent preparation was the PVP (Macrose) prepared for human infusions. In the previous series (experiments 101 and 102) the 0.5 ml. dose given to each mouse was approximately equivalent to an infusion of 1 l. in man, while in this series the total dose of 2.0 ml.

TABLE II

*Foam Cell Counts in Experiments Employing PVP (K-30) of Medium Molecular Weight*

| Experiment number | Total volume | Concentration | Quantity of PVP | Days after first injection | Days after final injection | Percentage of foam cells |
|---|---|---|---|---|---|---|
| | ml. | % | mg. | | | |
| 101 | 0.5 | 3.5 | 17.5 | 30 | 30 | 0.7 |
| 102 | 0.5 | 3.5 | 17.5 | 70 | 70 | 0.9 |
| 11 | 2.0 | 3.5 | 70 | 7 | 1 | 4.9 |
| 13 | 2.0 | 3.5 | 70 | 17 | 11 | 4.7 |
| 14 | 2.0 | 3.5 | 70 | 36 | 30 | 6.1 |
| 15 | 2.0 | 3.5 | 70 | 73 | 67 | 4.7 |
| 41 | 5.0 | 10.0 | 500 | 11 | 1 | 29.4 |
| 42 | 5.0 | 10.0 | 500 | 20 | 10 | 20.8 |
| 43 | 5.0 | 10.0 | 500 | 38 | 28 | 19.3 |
| 44 | 5.0 | 10.0 | 500 | 70 | 60 | 27.2 |
| 45 | 5.0 | 10.0 | 500 | 110 | 100 | 31.4 |
| 52 | 10.0 | 3.5 | 350 | 25 | 1 | 27.1 |
| 54 | 10.0 | 3.5 | 350 | 42 | 18 | 20.3 |
| 55 | 10.0 | 3.5 | 350 | 56 | 32 | 11.5 |
| 56 | 10.0 | 3.5 | 350 | 73 | 49 | 14.1 |
| 57 | 10.0 | 3.5 | 350 | 101 | 77 | 15.6 |

was approximately equivalent to 4 l. in man. It approaches the total estimated blood volume, perhaps falling short of this quantity by about 20 per cent. The total dose contained 70 mg. of PVP administered in four 0.5 ml. injections over a 6-day period. The animals were sacrificed at 1, 11, 30, and 67 days after the final injection. The foam cell counts, ranging from 4.7 to 6.1 per cent during the first 2 months after injection, were very similar to those after the same intervals when a 2.0 ml. volume of material of low molecular weight was used. However, since a 10 per cent solution of the K-20 PVP had been used, compared to a 3.5 per cent solution of the K-30 PVP, the difference in total quantity of PVP was almost 3 to 1. The foam cell counts at 1, 11, 30, and 67 days after injection were 4.9, 4.7, 6.1, and 4.7 per cent, respectively. The histologic appearance of the livers in the animals of this series was similar to that observed for experiments 131 to 134 in which the larger dose of the smaller molecules was administered. Rare, small, midzonal, and periportal nodules of foam cells occurred.

Two series of experiments were conducted in which massive total

quantities of material of medium molecular weight were administered. In the series comprising experiments 41 to 45 (Table II), a total of 500 mg. was administered. The animals received ten daily 0.5 ml. injections of a 10 per cent solution, and were sacrificed at 1, 10, 28, 60, and 100 days after the final injection. In the series comprising experiments 52, and 54 through 57, a total of 350 mg. was administered. These animals received twenty 0.5 ml. injections of the 3.5 per cent solution over a 24-day period. The total volume of 10.0 ml. was about four times the total blood volume of these animals. They were sacrificed at 1, 18, 32, 49, and 77 days after the last injection. It is important to consider in this series of experiments that the injections were given over a period of 24 days, so that 1 day post-injection is actually 25 days after the first injection was made. During this lengthy series of PVP injections many effects of earlier injections already were present at the time of the later injections. Tables I to III include the number of days after the first and the final injections for all the experiments reported.

The number of foam cells in the livers of the two groups of animals given the massive doses of PVP of medium molecular weight was moderately high. After the shorter post-injection intervals, the counts of the two groups were similar. After the later intervals the group which received 500 mg. still showed high counts while the animals which received 350 mg. showed a slight regression in the number of foam cells. In the first group (500 mg.) the percentage of foam cells ranged from 19.3 to 31.4, while in the second group (350 mg.) the range was from 11.5 to 27.1 per cent. The histologic appearances of the livers of the mice of both groups were similar in practically all respects. The more obvious characteristics were the greater abundance of foam cells and the development of larger vacuoles than had been seen in the experiments reported previously. Many bile ducts were completely surrounded by foam cells (Figs. 5 and 6).

A comparison of the foam cell counts resulting from the injection of massive quantities of PVP of low and medium molecular weight revealed that material of lower molecular weight resulted in less foam cell development. Despite the injection of approximately double the amount of PVP, material of low molecular weight resulted in about one half the percentage of foam cells induced by PVP of medium molecular weight.

*High Molecular Weight.* The results of experiments with the K-71 PVP preparation of high molecular weight are presented in Table III.

In experiments 91 and 92, mice were given single injections of this material of high molecular weight and sacrificed at 30 and 70 days

post-injection. Each mouse received 0.5 ml. of a 5.0 per cent solution, which contained 25 mg. of PVP. The percentage of foam cells after 30 and 70 days was approximately 1 per cent. The histologic appearance of the liver was similar to that in experiments 20, 101, and 102 in which there were no significant changes.

Another group of mice was injected with 0.5 ml. of a 5 per cent

TABLE III

*Foam Cell Counts in Experiments Employing PVP (K-71) of High Molecular Weight*

| Experiment number | Total volume | Concentration | Quantity of PVP | Days after first injection | Days after final injection | Percentage of foam cells |
|---|---|---|---|---|---|---|
| | ml. | % | mg. | | | |
| 91 | 0.5 | 5.0 | 25 | 30 | 30 | 1.2 |
| 92 | 0.5 | 5.0 | 25 | 70 | 70 | 0.9 |
| 121 | 2.0 | 5.0 | 100 | 7 | 1 | 4.1 |
| 122 | 2.0 | 5.0 | 100 | 17 | 11 | 34.7 |
| 123 | 2.0 | 5.0 | 100 | 26 | 20 | 22.1 |
| 65 | 2.0 | 5.0 | 100 | 50 | 44 | 21.7 |
| 124 | 2.0 | 5.0 | 100 | 82 | 76 | 20.0 |
| 125 | 2.0 | 5.0 | 100 | 132 | 126 | 17.7 |
| 66 | 5.0 | 5.0 | 250 | 59 | 21 | 42.2 |
| 63 | 6.5 | 5.0 | 325 | 23 | 3 | 39.2 |
| 64 | 10.0 | 5.0 | 500 | 45 | 3 | 68.3 |

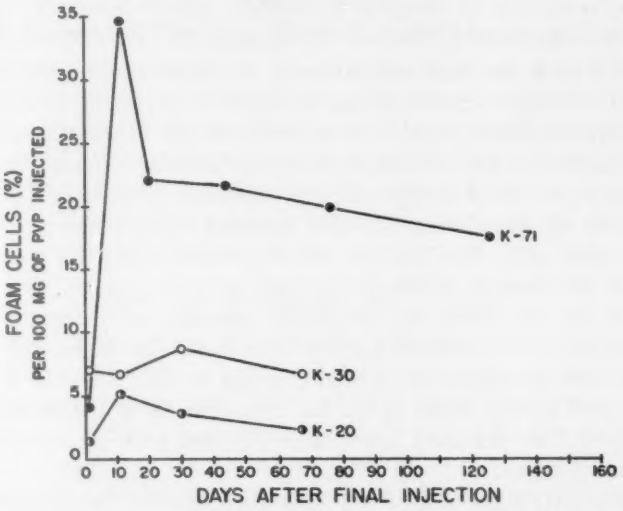
solution of this material on alternate days until four injections had been given. They thus received a total of 2.0 ml., which contained 100 mg. of PVP. They were sacrificed at 1, 11, 20, 44, 76, and 126 days after the final injection, and the foam cell counts were 4.1, 34.7, 22.1, 21.7, 20.0, and 17.7 per cent, respectively. Except for the count on the first day, the number of foam cells was moderately high. The mice of experiments 121 to 124 were injected intraperitoneally, while those of experiments 65 and 125 were injected subcutaneously.

After 11 days the foam cells were present in greatest number, and the histologic alteration was relatively extensive. After 20, 44, 76, and 126 days the foam cell counts were moderately high and the livers resembled those in the experiments in which massive quantities of PVP of medium molecular weight were used. Foam cells of the highly vacuolated type were especially abundant, and nodules were frequent throughout the lobules, especially in the areas of the portal triads. Leukocytic infiltration was relatively extensive, particularly around portal veins and bile ducts (Fig. 4).

Massive quantities of PVP of high molecular weight were employed in experiments 66, 63, and 64. They totaled 250, 325, and 500 mg., respectively. These experiments varied in total volume injected as well

as in the time after the last injection when the animals were sacrificed (Table III). In all of the livers, however, there was very extensive development of highly vacuolated foam cells and numerous aggregations of these cells into nodules in all regions. The foam cell counts in these three experiments ranged from about 40 per cent to almost 70 per cent.

Text-figure 2 illustrates the influence of molecular size in the induction of foam cell formation. Only the experiments in which a total of




Text-figure 2. Percentage of foam cells induced by 100 mg. doses of PVP solutions of low (K-20), medium (K-30), and high (K-71) molecular weight.

2.0 ml. of PVP was administered to each mouse are included in Text-figure 2. Since the concentration of PVP varied in the solutions containing different molecular weights, the foam cell counts have been figured on a basis of 100 mg. of PVP injected. The ratio of foam cell production induced by 100 mg. of PVP of low, medium, and high molecular weight appears to be approximately 1:2:6, which would indicate that foam cell production is a function of molecular size.

### *Results of Varying the Quantity of PVP Injected*

A direct relationship was shown between the foam cell counts and the quantity of PVP administered. Histologic examination after a single injection of 0.5 ml. revealed no changes of any significance. Tables I, II, and III illustrate the increased foam cell counts with increasing quantities of PVP preparations of low, medium, and high molecular

weight. Histologic examination revealed increases in nodule formation and vacuolization of foam cells with increasing quantities of PVP.

The data from experiments with material of medium molecular weight provide a typical example of the effect of the total quantity of PVP injected on the number of foam cells produced (Table II and Text-fig. 1). At approximately 30 days after injection of 17.5, 70, 350, and 500 mg. of PVP, the foam cell counts were 0.7, 6.1, 11.5, and 19.3 per cent, respectively (experiments 101, 14, 55, and 43).

## Discussion

### *The Sequence of Changes in the Liver after PVP Administration*

When PVP has been administered in quantity sufficient to cause extensive changes in the reticulo-endothelial tissues of the liver, the first sign of macrophagic activity is a swelling of Kupffer cells. In the most extensively altered liver tissues examined (experiment 64), 68.3 per cent of the lining cells were involved. The swollen cells first observed are mostly intermediate between normal lining cells and small foam cells. The Kupffer cell, instead of lying flat against the wall of the sinusoid, protrudes into the sinusoidal lumen. This expansion of the cell makes the cytoplasmic granules more readily visible. The nuclei of the swollen Kupffer cells, when cut in certain planes, appear less elongated, which might be due to the release of a tension which had caused them to lie flat. Another early manifestation of storage is the occasional appearance of foam cells in the periportal connective tissue.

These early signs of PVP deposition are followed by changes which are seen rarely in the liver of animals receiving small doses of PVP. A greater number of Kupffer cells become swollen, and the watery cytoplasmic vacuoles become larger and more numerous (Fig. 1). At this time, small clusters or nodules of foam cells appear in addition to the solitary foam cells observed heretofore (Figs. 1 and 2). At about the time that the small nodules of foam cells are forming in the sinusoids, there is an increase in the number of foam cells in the portal triad regions (Fig. 2). These cells appear to attain a highly vacuolated cytoplasm much sooner than the foam cells located elsewhere. Occasionally, almost the entire periportal area undergoes foam cell formation in animals given excessively large quantities of PVP (Fig. 5). The area immediately around the bile ducts is most consistently and severely affected (Figs. 5 and 6). The epithelial cells of the bile ducts are unchanged despite the alteration in the surrounding layers of connective tissue.

The possibility of a movement of macrophages loaded with PVP

from such locations as spleen, adrenal glands, and red bone marrow into the liver by way of the portal vein should be considered. The presence of migrating, PVP-laden macrophages in the periportal connective tissue of the liver would help to explain the large number of foam cells found in this area after large quantities of PVP are administered. Occasionally, this same area is infiltrated with leukocytes (Fig. 4).

Most of the changes described are not seen after "therapeutic" or "physiologic" doses of the 3.5 per cent solution of PVP (K-30) of medium molecular weight. When the quantity of this material, administered in several well spaced injections, does not exceed the total blood volume, very few foam cells develop, and these are almost all small, on the borderline between foam cells and normal lining cells. Nodules of foam cells rarely appear, nor does periportal infiltration of leukocytes occur (experiments 11, 13, 14, 15, and 16). A single injection of this preparation, approximately equivalent to a 1 l. human infusion, does not bring about any changes in the histologic picture of the liver of the mouse (experiments 101 and 102).

### *Foam Cell Development and Persistence*

The mechanism of the transformation of reticulo-endothelial cells of the liver into foam cells has not been explained adequately. Although the mechanism might vary slightly for different colloids, the fundamental change in the development of foam cells is the formation of watery vacuoles in the cytoplasm. Watery vacuoles might originate in several ways, as described by Trowell[7] for hepatic parenchymal cell vacuolization. The evidence in the case of reticulo-endothelial foam cells suggests that water enters the cell from outside. The main evidence is the direct relationship between the degree of vacuolization and the increase in cell size. A lining cell with barely visible cytoplasm lying flat against the wall of a sinusoid may increase so much in volume that it will appear as a large foam cell locally filling the sinusoid and distending it.

The success of PVP in the treatment of shock or circulatory collapse is due largely to its hydrophilic property, which enables it to maintain blood pressure long enough to permit the normal blood volume to be restored by physiologic processes. This capacity for binding water is apparently the basic mechanism responsible for the development of watery vacuoles, and, consequently, for the foam cells. There are several possible forms in which PVP might be ingested by reticulo-endothelial cells: (1) as discrete particles of PVP in the free state, (2) as products of PVP decomposition, (3) in combination with a

protein carrier. The second possibility is not a strong one since there is no evidence of decomposition of PVP in the body and much of the PVP in a solution injected intravenously will appear in the urine unchanged within the first 3 days.[8] The third possibility has much supporting evidence, particularly from electrophoresis and dye-binding experiments.[9]

Whatever the form of the phagocytized PVP, the particle exhibits its hydrophilic nature by accumulating water around it. The size as well as the number of particles in a cell might play an important part in the degree of vacuolization of the cytoplasm. Since the smaller molecules of PVP are found in the urine fairly early after injection and the larger molecules remain in the circulation for a long period of time, the larger ones should be found in the reticulo-endothelial system in greater abundance. Ravin *et al.*[10] concluded that phagocytosis by the reticulo-endothelial system as well as retention of PVP in the circulation is a function of molecular size.

The number of phagocytized particles would be expected to increase with time after injection, since many of the larger molecules of PVP remain in the circulation for days after injection. It is very likely that many of the smaller molecules which are not filtered through the glomeruli immediately after injection are also phagocytized by reticulo-endothelial elements.

The highly vacuolated condition of foam cells could be the result of several factors: the size of the PVP particles or a PVP-protein complex; the number of particles in the cytoplasm of the cell; the length of time the hydrophilic activity continues within the cytoplasm; and the coalescence of small vacuoles to form larger ones. Since a wide range of vacuolar sizes is observed in foam cells, from those barely visible under high microscopic magnification to those occupying almost the entire diameter of a swollen Kupffer cell, it seems reasonable to assume that submicroscopic vacuoles exist. This assumption leads to the conclusion that the development of vacuoles is a gradual process of which the rate depends on the four factors just given. This conclusion is strengthened by the fact that small doses of PVP do not bring about visible foam cell development, while larger doses as well as larger molecules and longer time intervals result in highly vacuolated foam cells.

Without a reliable histochemical method of demonstrating PVP in tissue sections, the relation of the deposits to the foam cell vacuoles is difficult to determine. It is possible that a fusion or compounding of particles takes place within the macrophages, resulting in large granular masses or plaques of PVP similar to the large granules or

plaques observed in the Kupffer cells by Easton[11] after administration of thorotrast. The size of the resulting watery vacuole might then be related to the size of the mass of PVP. During the process of histologic preparation of tissue sections, the highly soluble PVP would be dissolved and only the empty vacuoles would remain as evidence that it had been present in the living cell.

On the other hand, the compounding of PVP particles would not necessarily be a prerequisite to the formation of large vacuoles. It is possible that the PVP remains as separate particles within the macrophages. The size of the PVP molecule and the duration of its hydrophilic activity could then account for the size of the vacuole which exists after routine histologic procedures. A confluence of vacuoles might take place if the number were large enough.

Several possibilities concerning the fate of stored PVP exist: The particles may be egested from the cell to be excreted from the body or phagocytized by other macrophages; the particles may be retained by the cell throughout its life span and released only when the macrophage is destroyed or eliminated from the body; or, decomposition or depolymerization of the PVP molecule might occur which would result in products capable of being metabolized or excreted rapidly. Ravin *et al.*[10] concluded from their studies of the disposition of PVP tagged with $C^{14}$ that there is no metabolic degradation of PVP since there is no detectable radioactivity in the expired air. Whether the macrophages actually lose the stored PVP particles has not been determined.

### *The Effect of Molecular Size*

The number of foam cells and the extent of vacuolization in them varies with the average molecular size of the PVP preparations used. The same alterations which are produced by massive quantities of PVP of low molecular weight may be produced with smaller amounts of material of high molecular weight. In both cases highly vacuolated foam cells are observed, as well as scattered nodules of foam cells throughout the lobules and in the portal triads. The influence of molecular size in the induction of foam cell formation is illustrated in Text-figure 2. The ratio of foam cell production induced by 100 mg. of PVP of low, medium, and high molecular weight is approximately 1:2:6.

Naturally, in a unit weight of material of low molecular weight the total number of molecules is greater than in a similar weight of the larger molecules. If all molecules of PVP, regardless of size, were equally capable of inducing the formation of foam cells, the material of low molecular weight would be expected to have greater potential

for foam cell induction. The number of small PVP molecules is reduced greatly, however, by the process of biologic fractionation which takes place in the kidney, resulting in the earlier elimination of the smaller molecules.[10] This is not meant to imply that all the small molecules are eliminated before any are phagocytized by macrophages, but, on the contrary, the reticulo-endothelial system sometimes responds so rapidly to the injection of foreign colloids[12] that many small molecules of PVP would be expected to be phagocytized before reaching the renal filtering mechanism.

The foam cells observed after administration of large amounts of PVP of low molecular weight probably are not due entirely to phagocytosis of numerous small particles. Thrower and Campbell[13] pointed out that variability in average molecular weights of different lots of PVP exists, and the range of molecular weights in any sample of PVP is likely to be extensive. It appears, therefore, that the larger molecules in a solution of low molecular weight could be quite effective in the production of foam cells, particularly when a massive quantity of the material is administered.

*The Effect of Varying the Total Quantity of PVP Injected*

There are frequent reports in the literature that histologic changes do not occur until after a certain minimum dose of PVP is administered,[1,2,14,15] and that changes in the histologic appearance of various organs increase with increasing doses of PVP.[15-17] Conflicting reports appear regarding the size of the minimum dose which will bring about histologic changes. In the experiments presented here, PVP dosage is administered as follows: (1) a single injection of 0.5 ml. which corresponds to a human infusion of approximately 1 l., (2) a "maximum physiologic" dose consisting of four 0.5 ml. injections on alternate days and closely approximating the total blood volume of the animal, (3) a "massive" dose which corresponds to a volume two to five times the "maximum physiologic" dose.

Many authors have reported that "physiologic" doses of PVP do not result in storage phenomena in the liver,[18,18,19] but similar doses have been reported to result in alterations of the liver by Riedel and Zipf[20] and by Hüsselmann.[21] The results of our experiments with the PVP preparation of medium molecular weight are in accord with those of the former group of investigators when single injections of PVP are administered. This quantity of PVP does not change the histologic appearance of the liver. The "maximum physiologic dose," however, results in a mild reticulo-endothelial response which is characterized by the formation of about 5 per cent foam cells. These foam cells are predominantly very small. An occasional small nodule of foam

cells is seen, but the general microscopic appearance of the liver indicates only slight deviation from the normal.

Numerous investigators have administered "massive" doses of PVP to laboratory animals and all have found microscopic evidence of reticulo-endothelial alterations in the liver.[1,2,4,5,17,22-25] The experiments described in this paper provide additional evidence of this hepatic alteration after massive doses of PVP. Text-figure 1 clearly illustrates the effect of different quantities of PVP of medium molecular weight on the number of foam cells. The histologic effects of a larger total quantity of PVP are the greater abundance of foam cells, the greater degree of vacuolization, and the more frequent occurrence of nodules of foam cells.

The effect of varying the total quantity of PVP preparations of low and high molecular weight is shown in Tables I and III. A single injection of 0.5 ml., even when material of high molecular weight is employed, never results in a significant foam cell count. The unaltered histologic appearance of the liver indicates that there is no PVP storage. However, the studies of Steele *et al.*[26] and Ravin *et al.*,[10] using equivalent amounts of PVP labeled with radioactive isotopes, would suggest that, although the quantity of PVP injected does not result in any visible cytologic changes in the liver, the larger molecules are retained in the reticulo-endothelial tissues for some time.

Massive quantities of any of the three molecular weight preparations produce reticulo-endothelial changes, but the effect of massive doses of PVP of low molecular weight is slight compared to that of the effect of the solution of high molecular weight. From this observation we are led to conclude that although the total quantity of PVP injected is important in determining the degree of alteration resulting from tissue deposition, the size of the PVP molecules can be equally important. Although administration of large amounts of PVP of high molecular weight results in considerable retention in the liver, its presence does not interfere with normal liver function.[27]

## Summary

Low, medium, and high molecular weight fractions of polyvinylpyrrolidone (PVP) were injected into mice in doses which ranged from a single injection of 0.5 ml. to a series of twenty injections which totaled 10.0 ml. Reticulo-endothelial changes occurred only after a certain critical quantity of PVP was administered. Hepatic alterations involved the Kupffer cells and the histiocytes of the periportal connective tissue. Some macrophages were distended with vacuoles of various size which gave them the appearance of typical foam cells.

The percentage of foam cells among the total number of sinusoidal

lining cells of the liver was used as a criterion for evaluating the extent of histologic change due to PVP administration. The ratio of foam cell production induced by equivalent amounts of PVP of low, medium, and high molecular weight was approximately 1:2:6, which indicates that foam cell production is a function of molecular size.

The extent of histologic alteration in the macrophages of the liver increased with increasing quantities of PVP injected. A single injection, equivalent to 20 to 25 per cent of the total blood volume, did not result in a significant change from the normal appearance of liver tissue. Massive quantities of preparations of any of the three molecular weights produced hepatic alterations, but the effect of massive doses of PVP of low molecular weight was slight compared to the effect of the solution of high molecular weight.

It was shown that reticulo-endothelial changes after injections of PVP of low, medium, and high molecular weights depend on large molecules being present. The number of these large molecules determines the degree of storage.

I wish to thank Dr. J. Walter Wilson for his helpful guidance and constructive criticism throughout the course of this investigation.

## REFERENCES

1. Hartman, F. W. Tissue changes following the use of plasma substitutes. *A. M. A. Arch. Surg.*, 1951, **63**, 728–738.
2. Hartman, F. W. Fate and disposal of plasma substitutes. *Ann. New York Acad. Sc.*, 1952, **55**, 504–512.
3. Hueper, W. C. Macromolecular substances as pathogenic agents. *Arch. Path.*, 1942, **33**, 267–290.
4. Nelson, A. A., and Lusky, L. M. Pathological changes in rabbits from repeated intravenous injections of periston (polyvinyl pyrrolidone) or dextran. *Proc. Soc. Exper. Biol. & Med.*, 1951, **76**, 765–767.
5. Stern, K. Effect of polyvinylpyrrolidone on reticulo-endothelial storage. *Proc. Soc. Exper. Biol. & Med.*, 1952, **79**, 618–623.
6. Deane, H. W. A cytological study of the diurnal cycle of the liver of the mouse in relation to storage and secretion. *Anat. Rec.*, 1944, **88**, 39–65.
7. Trowell, O. A. The experimental production of watery vacuolation of the liver. *J. Physiol.*, 1946–47, **105**, 268–297.
8. Weese, H. Kreislaufregulation im Kollaps durch Blutflüssigkeitsersatzpräparate. *Wien. med. Wchnschr.*, 1951, **101**, 263–265.
9. Schubert, R. A new means of detoxicating the body by replacing the bile-liver system by the kidneys as organs of excretion of substances bound to artificial colloids. *Rev. Gastroenterol.*, 1950, **17**, 165–179.
10. Ravin, H. A.; Seligman, A. M., and Fine, J. Polyvinyl pyrrolidone as a plasma expander: studies on its excretion, distribution and metabolism. *New England J. Med.*, 1952, **247**, 921–929.
11. Easton, T. W. The role of macrophage movements in the transport and elimination of intravenous thorium dioxide in mice. *Am. J. Anat.*, 1952, **90**, 1–33.

12. Gabrieli, E. R., and Holmgren, H. Studies in the blockade of the reticulo-endothelial system. *Acta path. et microbiol. Scandinav.*, 1952, **31**, 205–211.

13. Thrower, W. R., and Campbell, H. Plasmosan. A synthetic substitute for plasma. *Lancet*, 1951, **1**, 1096–1099.

14. Weese, H. Blutersatzprobleme. *Deutsche med. Wchnschr.*, 1947, **72**, 555.

15. Fresen, O. Versuche mit Kollidon verschiedener Teilchengrösse. *Ärztl. Forsch.*, 1949, **3**, II Ref., 308.

16. Schallock, G. Anatomische Untersuchungen über das Schicksal von Blutersatzmitteln im Empfängerorganismus und der durch sie ausgelösten Reaktionen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1943, **108**, 405–451.

17. Bargmann, W. Über Milzveränderungen nach Zufuhr des Blutflüssigkeitsersatzes Periston. *Virchows Arch. f. path. Anat.*, 1947, **314**, 162–166.

18. Clerc, J. L. La polyvinylpyrrolidone ou subtosan, dans le traitement des hémorragies. *Ther. Umschau*, 1947–48, **4**, 149–153.

19. Korth, J., and Heinlein, H. Funktionelle und morphologische Untersuchungen über die Wirkung kolloidaler Blutersatzmittel unter besonderer Beachtung des Peristons. *Arch. f. klin. Chir.*, 1943–44, **205**, 230–282.

20. Riedel, G., and Zipf, K. Tierexperimentelle Untersuchungen über Blutersatzmittel. *Arch. f. exper. Path. u. Pharmakol.*, 1944, **203**, 25–33.

21. Hüsselmann, H. Speicherungserschienungen beim Menschen nach Periston. *Klin. Wchnschr.*, 1952, **30**, 801–808.

22. Müller, W. Speicherungsversuche mit Kollidon. *Zentralbl. f. allg. Path. u. path. Anat.*, 1948–49, **84**, 292–293.

23. Pellerat, J.; Maral, R., and Murat, M. Étude histologique des viscères de cobayes après injections massives de polyvinylpyrrolidone (subtosan). *Ther. Umschau*, 1948, **4**, 153–155.

24. Ammon, R., and Braunschmidt, G. Das Schicksal des Peristons im Organismus. *Biochem. Ztschr.*, 1949, **319**, 370–377.

25. Ammon, R., and Müller, W. Der Einfluss hoher Peristongaben auf den Kaninchenorganismus unter besonderer Berücksichtigung der Speicherorgane. *Deutsche med. Wchnschr.*, 1949, **74**, 465–467.

26. Steele, R.; Van Slyke, D. D., and Plazin, J. The fate of intravenously administered polyvinylpyrrolidone. *Ann. New York Acad. Sc.*, 1952, **55**, 479–484.

27. Frommer, J. Effect of polyvinylpyrrolidone (PVP) on mouse liver function. *Proc. Soc. Exper. Biol. & Med.*, 1954, **86**, 567–569.

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[ *Illustrations follow* ]

## LEGENDS FOR FIGURES

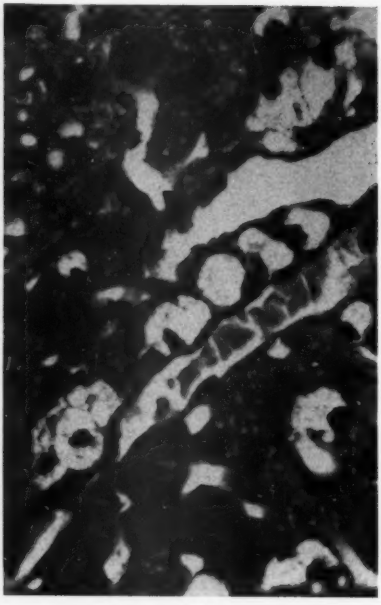
All sections were stained with Delafield's hematoxylin and eosin.

FIG. 1. Extensively distended Kupffer cells contain vacuoles which give them the appearance of typical foam cells. Aggregates of foam cells appear as small nodules. × 615.

FIG. 2. Single foam cells and foam cell nodules in the region of the portal triad. × 615.

FIG. 3. Two nodules of foam cells in midzonal region of lobule. Very large vacuoles are present in a few foam cells. × 1,260.

1



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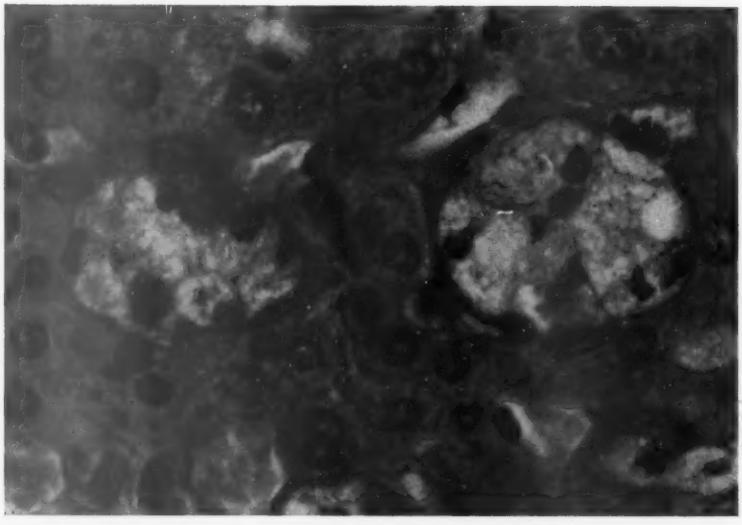
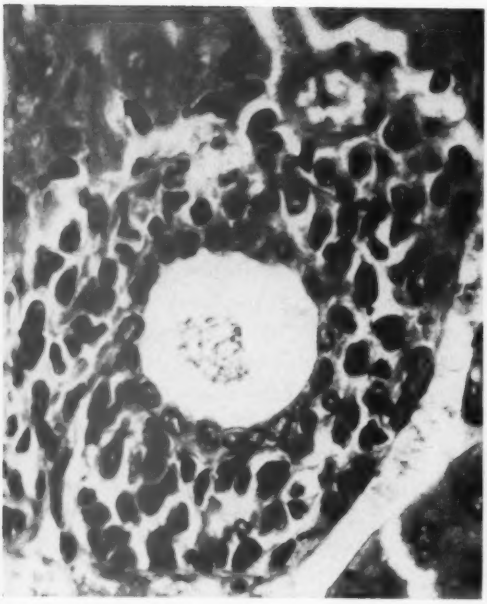


3

FIG. 4. Infiltration of leukocytes in the periportal area, particularly in the region surrounding the bile duct. × 615.

FIG. 5. Extensive foam-cellular formation in the region of the portal triad. × 140.

FIG. 6. A portion of Figure 5 more highly magnified. Bile duct completely surrounded by foam cells. The epithelial cells of the bile duct appear to be normal. × 615.



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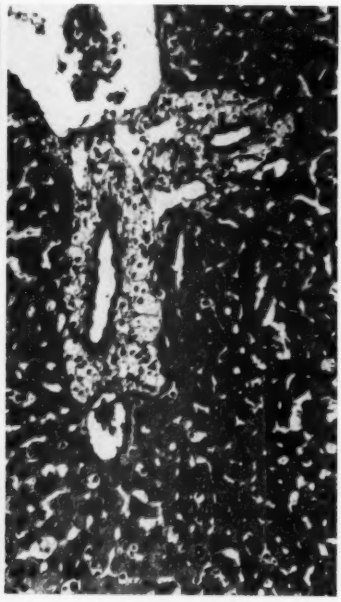

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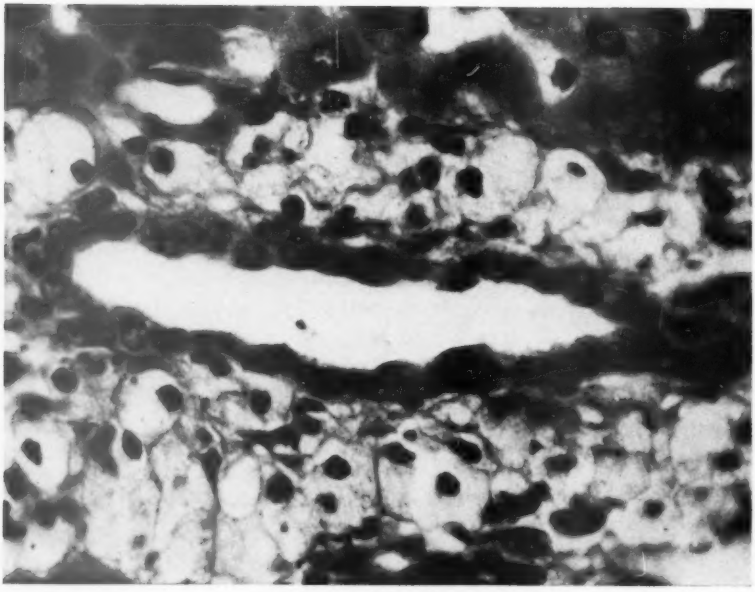

6

ANALYTICAL PATHOLOGY

# III. New Observations on the Pathogenesis of Glomerulonephritis, Lipid Nephrosis, Periarteritis Nodosa, and Secondary Amyloidosis in Man *

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The present work establishes beyond a reasonable doubt that gamma globulins are localized in the glomerular lesions of lipid nephrosis and of glomerulonephritis in man. Evidence is presented also that gamma globulins are localized in the glomerular lesions of renal amyloidosis and in the vascular lesions of periarteritis nodosa in man. It had already been found[1-4] that globulins are localized in the glomerular lesions in two forms of experimental glomerulonephritis which histologically simulate the human renal disease. By demonstrating that proteins of the type known to include antibodies are localized at the sites of the lesions, the present and the preceding observations fulfill a requirement essential for the immuno-allergic pathogenesis of these several diseases of man and experimental animals, a concept of pathogenesis widely discussed during the last half century[5,6] and supported heretofore only by indirect evidence. The success in the present investigations, which utilize a new histopathologic application[2] of an immunohistochemical method,[7] suggests that the pathogenesis of a variety of diseases, among them rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus, and other so-called collagen diseases, and certain types of purpura, hemolytic anemia, and encephalomyelitis, may be investigated by this method. Moreover, if it is accepted that the pathogenic mechanism in glomerulonephritis can now be studied by this means, then an exploration can be made of preventive and therapeutic measures by looking for those agents which inhibit the mechanism in the experimental animal. Agents hav-

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ing anticomplementary activity and capable of influencing antigen-antibody reactions that require complement appear to be among the more promising in this respect.

## Materials and Methods

*Fluorochroming Reagent.* The fluorochroming reagent for the demonstration of localizing globulins in frozen sections of human tissues was prepared by coupling rabbit antibodies against human globulins with fluorescein isocyanate, in accordance with the method of Coons and Kaplan.[7] For the most part the analytical procedures have been described elsewhere.[1,2]

The starting material for the preparation of rabbit antisera against human globulins was immune human globulins (Armour). Rabbits were given five intramuscular injections, each consisting of 10 mg. of human globulins in Freund adjuvant, and the immunized animals were bled by heart puncture on the fourth, fifth, and sixth weeks. The globulin fractions of the rabbit antisera were obtained by precipitation at a 25 per cent concentration of ethanol in accordance with the Cohn method.[8] The globulin precipitates were reconstituted in isotonic saline solution, dialysed, frozen, and stored until needed.

The rabbit globulins were coupled with fluorescein isocyanate, prepared by Dr. Malcolm Siegel in accordance with the method of Coons and Kaplan.[7] Their method also was followed throughout the coupling procedure, and 0.05 mg. of fluorescein amine was used for each milligram of protein. The coupled proteins were dialysed against phosphate buffer, pH 7, for 1 week; cleared by centrifugation; preserved with merthiolate, 1:10,000; and stored in the refrigerator until needed. Aliquots of the coupled proteins were further purified prior to use by treatment with 200 mg. of mouse liver powder per 1 ml. of aliquot and then by filtration through a Seitz filter.[7] The fluorescein-labeled rabbit antibodies against human globulins so purified are referred to as the fluor, and their use as the fluorochroming procedure, in this paper.

By means of the gel diffusion method of Ouchterlony,[9] as modified,[10] Dr. Leonhard Korngold demonstrated that the fluor used in this work contained rabbit antibodies against pure human $\gamma_2$-globulin as well as against a component of the human $\beta$-globulins. No antibody against human albumin was detected. Aliquots of the fluor were reacted with an excess of pure human $\gamma_2$-globulin, obtained from Dr. Korngold, who had isolated the protein by using zone electrophoresis in starch, so that there was complete absorption of antibodies against $\gamma_2$-globulin as shown by precipitin ring tests; whereupon those aliquots of the fluor

no longer imparted specific fluorescence to the sites of the lesions when used in the fluorochroming procedure. This fact, together with additional data which will be presented concerning immunologic specificity, proves that the standard fluorochroming procedure used in this investigation specifically displays sites of localization of human gamma globulin.

*Freezing and Storage of Tissue Blocks.* Tissue blocks, about the size of the object holder of the microtome and 2 to 4 mm. thick, were wrapped in glassine paper, placed on a flat surface of dry ice, and covered with crushed dry ice so as to be rapidly frozen. The frozen blocks were wrapped in multilayers of Saran Wrap and then in pure aluminum foil and stored at −10° to −18° C. until used.

*Preparation and Storage of Frozen Sections.* Frozen sections of tissue were cut at about 8 to 10 $\mu$ by the method of Bush and Hewitt[11] which utilizes Kodak frozen section film for the support and the transport of the section. However, in the present work, after thawing the section, the supporting film was immersed obliquely in a container of water so that the section floated off and remained on the surface of the water. The section was then lifted up on a microscopic slide, excess water was removed from the slide with absorbent paper, and further dehydration was carried out in a vacuum desiccator for a period of 20 to 30 minutes. The mounted and dried sections were stored over a desiccant at −10° to −18° C. until used.

*Standard Fluorochroming Procedure.* The dried and mounted frozen sections were washed in five changes of isotonic saline solution containing 0.01 M phosphate buffer at pH 7.0, a procedure requiring a total time of about 15 minutes. The slides were drained briefly and excess fluid around the sections was removed by absorbent paper. The sections were covered with the fluor by means of a dropping pipette, and the slides were covered with a Petri dish which had been lined with filter paper and moistened with buffer-saline solution to prevent evaporation. The fluorochroming reaction was carried out at room temperature for 45 minutes. At the end of this time, the sections were washed in three changes of buffer-saline solution, requiring a total time of about 3 minutes. The slides were drained, excess fluid was removed with absorbent paper, and the sections were washed with glycerin medium containing 10 per cent of buffer-saline solution and then mounted in a similar glycerin medium under a coverslip sealed with paraffin.

*Immunologic Specificity.* The specificity of the fluorochroming procedure was demonstrated according to the criteria of Coons and Kaplan[7] by (a) specific inhibition of the reaction with uncoupled rabbit

antibodies against human globulins, homologous normal rabbit serum failing to do so under identical conditions; and by (b) specific removal of antibody from the fluor, and as a consequence inhibition of the fluorochroming reaction, by precipitation with human $\gamma_2$-globulin. In addition, the specific localization of globulins in certain pathologic lesions described herein was confirmed by using another preparation of fluorescein-labeled rabbit antibodies against human globulins kindly provided by Dr. Wilbur Noyes; whereas fluorescein-labeled rabbit antibodies against rat antibodies failed to impart any specific fluorescence to these lesions.

For the specific inhibition of fluorescence, the sections, after the customary washing with buffer-saline solution, were incubated in duplicate, one with uncoupled rabbit antibodies against human globulins, the other for an identical time with normal rabbit serum. At the completion of the period of incubation each section of the pair was washed with buffer-saline solution and then treated with the fluor under identical conditions. The uncoupled rabbit antibodies against human globulins inhibited the specific fluorescence, whereas the normal rabbit serum did not.

*Fluorescence Microscopy and Photography.* The essential details have been described elsewhere.[1] The method of photographic photometry has been used as before to compute the relative intensities of the characteristic green-yellow fluorescence imparted by the fluor. With suitable filters in the microscope, this characteristic fluorescence was readily distinguished from the blue-white, primary fluorescence of tissue proteins, especially that of elastic and collagenous fibers, and from the varicolored primary fluorescence of lipid substances. The glomerular-tubular ratios of fluorescence intensities as used throughout this paper pertain to the characteristic green-yellow fluorescence imparted by the fluor. Light intensities were not measured in regions of tissues where primary fluorescence might influence the result.

*Histologic Studies.* Tissue blocks were fixed in 10 per cent neutral formalin. Paraffin sections were stained routinely with hematoxylin and eosin and with the periodic acid-Schiff stain. Special stains for fat, elastic tissue, and collagenous tissue were used on occasion.

## PATHOLOGIC MATERIAL

### *Control Kidneys*

Kidneys to be used as controls were obtained from a group of 16 cases, of which 7 were males and 9 females, with a range of ages from 6 to 71 years and a mean age of 53 years. Fourteen renal specimens were obtained at necropsy, from 1 to 20 hours post mortem, on cases

with various forms of disseminated cancer, principally carcinoma, but including one example each of lymphosarcoma, reticulum cell sarcoma, and acute leukemia. The majority of these cases also had some form of terminal infection, most commonly bronchopneumonia. Two kidneys were surgical specimens obtained by nephrectomy, one from a patient with renal cell carcinoma and the other, a non-functioning, obstructed kidney, from a patient who had previously had cutaneous ureterostomy and complete pelvic exenteration for advanced carcinoma of the rectum.

Three patients had clinical histories of moderate diastolic and systolic hypertension. Two patients were in a state of shock prior to death. Urinanalysis had disclosed albumin, from a trace to 1 plus, in 5 cases, and hematuria in the patient with renal cell carcinoma. The blood urea nitrogen was normal in 11 of 12 cases examined, and the sole elevation was explainable on the basis of prerenal azotemia.

There were no histopathologic lesions of note in the kidneys of 3 cases, while the principal renal lesions in the remaining 13 cases were: arterial nephrosclerosis, 5 cases; acute suppurative pyelonephritis, 4 cases, one also being the site of metastatic epidermoid carcinoma; arteriolar nephrosclerosis, 2 cases; parenchymal nephrocalcinosis, one case; and renal cell carcinoma, one case. Representative frozen sections of these kidneys were treated with the fluor, and the results are discussed herein as those obtained on the control kidneys, or simply, the controls.

*Lipid Nephrosis and Glomerulonephritis*

There were 9 cases of lipid nephrosis and glomerulonephritis, of which 2 were in children. The kidneys were obtained at necropsy performed 2 to 24 hours after death.

One case, occurring in a boy 5 years of age, had many of the features of so-called lipid nephrosis with the nephrotic syndrome. The disease had occurred with an abrupt onset 11 months prior to death, with no known antecedent illness, and was characterized by generalized edema, essentially normal blood pressure, 4 plus proteinuria (with microscopic hematuria on occasion), decreased serum albumin, reversal of the serum albumin-globulin ratio, an elevated serum cholesterol, and normal blood urea nitrogen levels. At necropsy the kidneys were 50 per cent larger than normal, and were smooth and pale tan. Microscopically, the glomeruli generally were enlarged, and there was some increase in the number of endothelial cells, but the most conspicuous feature was thickening of the glomerular capillary walls (Fig. 1), so that there was partial and, on occasion, complete occlusion of some of the capillary lumina. A few glomeruli were

undergoing fibrosis. The tubules displayed some degeneration and atrophy, their epithelium contained stainable, although not strongly birefringent, fat, and in their lumina were protein casts. Focal accumulations of lymphocytes and plasma cells were present in the interstitium. The blood vessels were essentially normal. Histologically, this case was classified[12,13] as lipid nephrosis (chronic membranous glomerulonephritis).

While similar to the preceding case in the clinical prominence of the nephrotic syndrome, the second case of renal disease in childhood, in a boy 4 years of age, was dissimilar in the following respects: the disease followed a streptococcal sore throat; gross hematuria occurred at the onset, 15 months prior to death; thereafter, 4 plus proteinuria and generalized edema developed; and the blood pressure and blood urea nitrogen were significantly elevated, especially terminally. At necropsy the kidneys were nearly twice the normal weight. Microscopically, the glomeruli displayed various stages of structural alteration. There was an increase in endothelial cellularity and thickening of the capillary walls in some, but many glomeruli had undergone fibrosis (Fig. 2), leading in some to complete obliteration. The tubules were alternately atrophic and hyperplastic in areas, their lumina contained protein casts, and their lining epithelium contained cytoplasmic fat. The interstitium was infiltrated focally with lymphocytes, and the blood vessels were not remarkable. Histologically, this case was classified as chronic glomerulonephritis (chronic membranous and sclerosing glomerulonephritis) with the nephrotic syndrome.

The remaining 7 cases occurred in adults. Two cases, in females 60 and 70 years of age, had rather similar clinical and pathologic features which will be presented in brief summary. Hematuria, known to be present for 6 to 8 weeks prior to death, 2 to 3 plus proteinuria, azotemia with the blood urea nitrogen rising to or above 100 mg. per 100 ml. terminally, and hypertension were common to both. At necropsy, in each case the kidneys were somewhat enlarged, and there were petechial hemorrhages measuring about 1 mm. in diameter throughout the cortex. Microscopically, there were several structural alterations in the glomeruli (Fig. 3): increased endothelial cellularity, hemorrhage into Bowman's space, fibrous and cellular crescents, focal areas of capillary necrosis, and fibrosis. The tubules showed degeneration and atrophy, and their lumina contained red blood cells and protein or cellular casts. Focal accumulations of lymphocytes and plasma cells were present in the interstitium. There was arterial and arteriolar sclerosis. These cases were classified histologically as acute and sub-

acute diffuse glomerulonephritis — acute diffuse hemorrhagic and subacute (crescentic) glomerulonephritis.

The renal diseases in 4 cases represented incidental findings at necropsy in 2 males, 27 and 53 years of age, and in two females, 41 and 72 years of age. The younger male had metastatic malignant melanoma; the older, metastatic bronchogenic carcinoma, diabetes mellitus, and a history of penicillin hypersensitivity and generalized urticaria occurring 6 weeks prior to death. The younger female had metastatic mammary carcinoma; the older, portal cirrhosis of the liver and positive serologic tests for syphilis. Mild hypertension existed in both females. Proteinuria was present in the younger male and the older female. Other laboratory data were non-contributory to the present discussion. Aside from the stated diseases in these cases, some form of infection was found to be a frequent accompaniment at necropsy. The kidneys were not remarkable grossly at necropsy. Microscopically, the cases were classified as acute diffuse glomerulonephritis, 3 cases being the membranous type (Fig. 4) and one the exudative type.

A final case of acute glomerulonephritis occurred in association with periarteritis nodosa and will be discussed in that connection.

*Periarteritis Nodosa with Acute Glomerulonephritis*

One case of periarteritis nodosa with acute glomerulonephritis, occurring in a 69-year-old male, was studied. The kidney was obtained at necropsy performed 24 hours after death. The patient had a history of unexplained hematuria antedating the present admission by a substantial period. A drug rash, thought to be due to penicillin hypersensitivity, occurred 2 months prior to death. Moderate terminal hypertension, hematuria, proteinuria, and terminal azotemia, with the blood urea nitrogen rising to 179 mg. per 100 ml., were features of the disease. At necropsy there were mottled red and yellow areas of hemorrhage and necrosis in the kidneys. Microscopically, the lesions of necrotizing angiitis, as well as healed stages of the process, were present in the arterial system, including the range of calibers from arterioles to medium-sized arteries of the kidney (Fig. 5), as well as of the heart, lungs, liver, spleen, gastro-intestinal tract, adrenal glands, prostate, bladder, and testicles. The manifold vascular lesions included fibrinoid necrosis and acute exudative inflammation, with a predominance of neutrophilic and eosinophilic polymorphonuclear leukocytes; involvement by necrosis and inflammation of one or all coats of the arterial wall, with predilection for the media, and extension of

the process to the periadventitial tissue; destruction of the elastic lamellae, and segmental necrosis and aneurysmal dilatation of the arterial walls; and hemorrhage, thrombosis, infarction, and organization. The histologic classification of the vascular disease was periarteritis nodosa. In addition, there was acute diffuse glomerulonephritis with necrotizing glomerulonephritis (Fig. 6), which we presume was causally, rather than casually, related to the periarteritis nodosa.

### *Renal Amyloidosis*

One example of renal amyloidosis was studied. The kidney was obtained at necropsy performed 3 hours after death. The patient had a history of rheumatism of 10 years' duration. He had been treated with colloidal gold and had developed hypersensitivity to the drug, as shown by generalized skin rash, several months prior to death. He had had an abdominoperineal resection of a rectal adenocarcinoma 2 years prior to death, and the occurrence of bleeding from the colostomy and diarrhea had led to the present admission. Other clinical and laboratory data were non-contributory to the present discussion. At necropsy the kidneys, liver, and spleen were enlarged. Microscopically, there was amyloidosis of the kidneys (Fig. 7), liver, spleen, adrenal glands, and lymph nodes. The amyloid deposits stained with Congo red. Extensive interstitial pneumonitis, bilateral bronchopneumonia, acute ileocolitis, and acute peritonitis were present. There was no residual carcinoma.

## Results

The results are presented in standard photomicrographs, Figures 1 to 7, to which reference has already been made; fluorescence photomicrographs, Figures 8 to 26; Text-figures 1 and 2; and Tables I to V.

### *Control Kidneys*

When thin, unfixed frozen sections of human kidneys in the control group were treated with the standard fluorochroming procedure for the demonstration of localizing human gamma globulins, the intensity of the characteristic yellow-green fluorescence imparted by the fluor was observed to be about equal in glomeruli and tubules (Figs. 8 to 12). The average glomerular-tubular ratio of fluorescence intensity as determined by the photometric analysis of 362 glomeruli in the control group of 16 cases was found to be 1.2, and the confidence interval, which statistically includes 99 per cent of the estimates of the average, 1.1 to 1.3, Table I. When the influence of age was examined, it appeared that the glomerular-tubular ratio of intensity was somewhat

increased beyond the fourth decade of life. The frequency distribution of values for the glomerular-tubular ratio of intensity in the control group is given in the left half of Text-figure 1 in terms of the cumulative percentage of the determinations. There it may be seen that about 50 per cent of the determinations of the glomerular-tubular ratio of

TABLE I

*Glomerular-Tubular Ratio of Fluorescence Intensity in Sections of Human Kidneys (Control Group) Treated with the Fluorochroming Procedure for Demonstration of Localizing Human Gamma Globulins*

| Age (decade) | No. of cases | Ratio of fluorescence intensity | | |
|---|---|---|---|---|
| | | Av. | Ci. | N. |
| 1st | 2 | 1.0 | 0.9–1.1 | 49 |
| 4th | 1 | 1.0 | 0.9–1.1 | 19 |
| 5th | 4 | 1.3 | 1.2–1.4 | 103 |
| 6th | 1 | 1.1 | 1.0–1.2 | 33 |
| 7th | 6 | 1.4 | 1.3–1.5 | 129 |
| 8th | 2 | 1.3 | 1.2–1.4 | 29 |
| All cases | 16 | 1.2 | 1.1–1.3 | 362 |

Av. = average.
Ci. = confidence interval which includes 99 per cent of the estimates of the average.
N. = number of measurements.

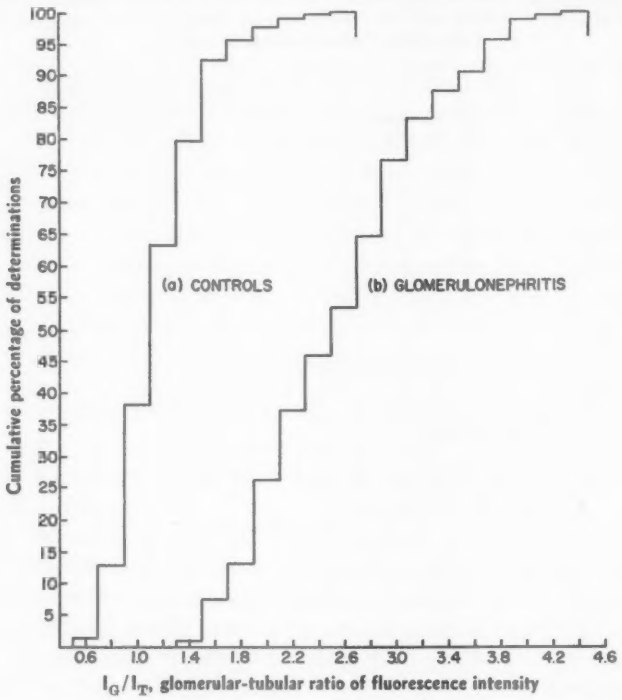
intensity in the control group fall either below or above 1.2 and that about 95 per cent fall below 1.8.

That the preliminary washing of the sections, as described under methods, sufficed to remove most, if not all, of the unbound (free) plasma globulins in the tissue sections was shown in 2 cases by the failure of thorough saline perfusion of an intact kidney to effect a lower glomerular-tubular ratio of fluorescence intensity than that observed in the unperfused kidney. In fact, because of some loss of tubular mass in the perfusion fluid, the glomerular-tubular ratio of intensity was slightly increased in the perfused kidney, although the absolute glomerular intensity was not substantially changed.

As stated in the discussion of materials, the control group was composed of 16 cases, about equally divided between sexes, with a range of ages from 6 to 71 years and a mean age of 53 years. There were no histopathologic lesions of note in the kidneys of 3 cases, while the principal renal lesions in the remaining 13 cases were arterial nephrosclerosis, 5 cases; acute suppurative pyelonephritis, 4 cases; arteriolar nephrosclerosis, 2 cases; parenchymal nephrocalcinosis, one case; and renal cell carcinoma, one case.

*Lipid Nephrosis and Glomerulonephritis*

When thin, unfixed frozen sections of kidneys from cases of lipid nephrosis and glomerulonephritis were treated with the standard fluorochroming procedure for the demonstration of localizing human gamma globulins, the intensity of the characteristic yellow-green fluorescence imparted by the fluor was observed to be strikingly increased




Text-figure 1. Glomerular-tubular ratio of intensity of characteristic fluorescence, plotted on the horizontal axis, for fluor-treated sections of human kidneys in (a) the control group and in (b) the glomerulonephritis group. The vertical axis represents the cumulative percentage of determinations, where all the determinations in a group constitute 100 per cent. The higher ratio of fluorescence intensity in the glomerulonephritis group, due to the localization of gamma globulins, is clearly shown.

in the glomeruli, both relatively and absolutely in comparison with the tubules (Figs. 13 to 15 and 18 to 23), or with any other histologic structure in the section, or with glomeruli in the kidneys of the control group (Figs. 8 to 12). It must be kept in mind that black and white illustrations do not, as did the full-color image in the fluorescence microscope, display the characteristic differences in color imparted by the fluor (yellow-green) from that possessed by cellular proteins and

collagenous or elastic fibers as intrinsic fluorescence (generally, blue-white). The descriptions and the quantitative data in this paper pertain, as previously stated, only to the characteristic fluorescence imparted by the fluor at the sites of gamma globulin localization.

In lipid nephrosis (chronic membranous glomerulonephritis) of childhood, the characteristic fluorescence occurred variously in the entire glomerular tuft (Fig. 13), in a portion of a glomerulus (Fig. 14), or only in a few capillaries of a glomerulus (Fig. 15). In chronic glomerulonephritis (chronic membranous and sclerosing glomerulonephritis) of childhood accompanied by the nephrotic syndrome, the characteristic fluorescence occurred in glomeruli or portions of glomeruli that had not undergone fibrosis (Fig. 18). Similarly, the glomerular tufts, but not the fibrous and cellular crescents, were the sites of characteristic fluorescence in two cases of combined acute and subacute diffuse glomerulonephritis in adults (Fig. 19).

The characteristic fluorescence occurred to a greater or lesser degree in practically all of the glomeruli from 4 adults with acute diffuse glomerulonephritis (Figs. 20 to 22). The localization of the characteristic fluorescence in the glomerular capillary basement membranes was notable in all cases and strikingly portrayed in some (Fig. 21).

The characteristic fluorescence was present in some of the glomeruli in a case of acute glomerulonephritis (Fig. 23) in an adult with periarteritis nodosa, about which more will be said subsequently.

The immunologic specificity of the fluorochroming procedure was demonstrated by the effectiveness of prior treatment of sections with uncoupled rabbit antibodies in completely inhibiting the specific fluorescence (Fig. 16). Under these circumstances the uncoupled rabbit antibodies occupy the receptive sites on the localized human globulins and prevent the fluorescein-labeled antibodies from imparting the characteristic fluorescence to these sites. The glomeruli then can scarcely be seen in cases of glomerulonephritis or in lipid nephrosis—actually one of the brighter glomeruli is illustrated in Figure 16 in order to facilitate identification—but the primary and the non-specific fluorescence of the tubules and of the other histologic structures persists undiminished. On the other hand, the prior treatment of sections with normal rabbit serum in no way interferes with the subsequent fluorochroming reaction, and the glomeruli fluoresce characteristically, appearing just as they do in Figures 13 and 18 to 23.

A final proof of immunologic specificity was obtained by the specific removal of antibody from aliquots of the fluor by precipitation with human $\gamma_2$-globulin and, as a consequence, inhibition of the fluorochroming reaction. A picture exactly similar to that in Figure 16 occurred

when kidney sections from a case of lipid nephrosis, as well as from cases of glomerulonephritis, were treated with such aliquots of the fluor, now lacking rabbit antibodies against human gamma globulins. These aliquots, unlike the original fluor, no longer imparted characteristic fluorescence to the glomerular sites of globulin—and we may now say, gamma globulin—localization.

Ancillary information was obtained by two additional tests. The characteristic fluorescence was imparted to the glomerular lesions by a preparation of fluorescein-labeled rabbit antibodies against human globulins that was obtained from another laboratory, thus attesting to the reproducibility of the results. Finally, a preparation of fluorescein-labeled rabbit antibodies against rat globulins, shown to impart characteristic fluorescence to the glomerular lesions in experimental glomerulonephritis in rats,[4] failed to display any characteristic fluorescence whatsoever in the glomerular lesions in human lipid nephrosis (Fig. 17) and glomerulonephritis. It scarcely need be said that the only difference between fluorescein-labeled rabbit antibodies against *rat* globulins and those against ***human*** globulins is shown by their reaction in the presence of a specific antigen.

### *Quantitative Data*

Quantitative data are summarized in Tables II and III and in Text-figures 1 and 2. The average glomerular-tubular ratio of fluorescence intensity as determined by the photometric analysis of 183

TABLE II

*Glomerular-Tubular Ratio of Fluorescence Intensity in Sections of Human Kidneys (Lipid Nephrosis and Glomerulonephritis) Treated with the Fluorochroming Procedure for the Demonstration of Localizing Human Gamma Globulins*

| Type of diffuse glomerulonephritis | No. of cases | Ratio of fluorescence intensity | | |
|---|---|---|---|---|
| | | Av. | Ci. | N. |
| In children | | | | |
| Lipid nephrosis | 1 | 2.8 | 2.5–3.1 | 37 |
| Chronic | 1 | 1.9 | 1.7–2.1 | 17 |
| In adults | | | | |
| Acute and subacute | 2 | 2.7 | 2.4–3.0 | 33 |
| Acute | 4 | 2.6 | 2.4–2.8 | 77 |
| Acute, with periarteritis nodosa | 1 | 2.6 | 2.2–3.0 | 19 |
| All cases | 9 | 2.5 | 2.2–2.8 | 183 |

Av. = average.
Ci. = confidence interval which includes 99 per cent of the estimates of the average.
N. = number of measurements.

glomeruli in the 9 cases of lipid nephrosis and glomerulonephritis was found to be 2.6 and the confidence interval, 2.4 to 2.8 (Table II). There was a statistically significant increase in the ratio, both collectively and individually by case, in the glomerulonephritis group relative to the control kidneys (Table I) in which the average ratio was 1.2

TABLE III

*Glomerular-Tubular Ratio of Fluorescence Intensity; Effectiveness of Prior Treatment of Sections with Uncoupled Rabbit Antibodies Against Human Globulins in Inhibiting the Specific Fluorescence Imparted by the Fluorochroming Procedure*

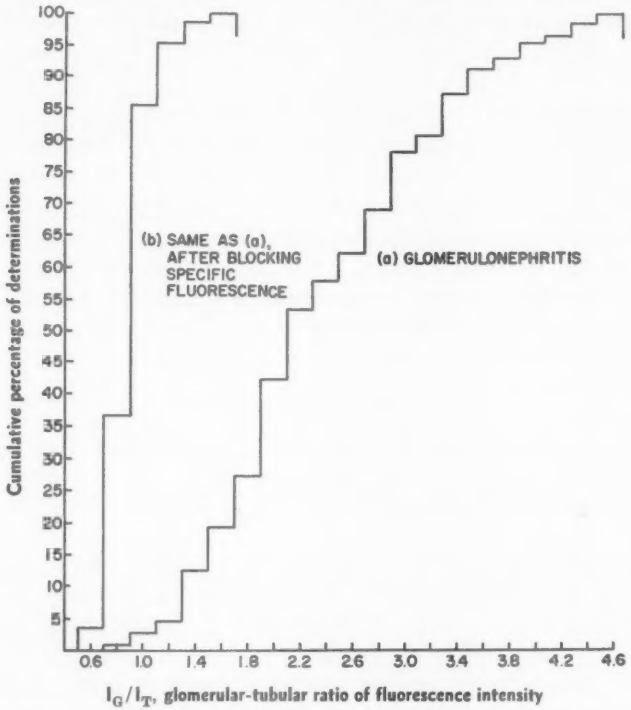
| Type of diffuse glomerulonephritis | Section treatment and ratio of fluorescence intensity | | | | | |
|---|---|---|---|---|---|---|
| | Normal rabbit serum | | | Uncoupled rabbit antibodies | | |
| | Av. | Ci. | N. | Av. | Ci. | N. |
| In children | | | | | | |
| Lipid nephrosis | 2.9 | 2.5–3.3 | 16 | 0.8 | 0.6–1.0 | 25 |
| Chronic | 1.3 | 1.1–1.6 | 10 | 0.9 | 0.6–1.2 | 9 |
| In adults | | | | | | |
| Acute and subacute | 2.6 | 2.2–3.0 | 20 | 1.4 | 1.2–1.6 | 7 |
| Acute | 2.4 | 2.0–2.8 | 60 | 1.0 | 1.0–1.0 | 80 |
| Acute, with periarteritis nodosa | 2.4 | 1.8–3.0 | 6 | 1.1 | 1.0–1.2 | 10 |
| All cases | 2.3 | 1.9–2.7 | 112 | 1.0 | 0.9–1.2 | 131 |

Av. = average.
Ci. = confidence interval which includes 99 per cent of the estimates of the average.
N. = number of measurements.

and the confidence interval, 1.1 to 1.3. The dissimilarities of the two groups are further illustrated in Text-figure 1. There it may be seen that about 50 per cent of the determinations of the glomerular-tubular ratio of intensity fall either below or above 2.6 in the glomerulonephritis group (b), and 50 per cent occur below or above 1.2 in the control group (a); and that about 85 per cent of the determinations occur above 1.8 in the glomerulonephritis group, whereas only 5 per cent of the values are in excess of 1.8 in the control group.

Additional quantitative information is provided in Table III and Text-figure 2. The average glomerular-tubular ratio of fluorescence intensity in the glomerulonephritis group was found to be 2.5, and the confidence interval, 2.3 to 2.7 (Table III) in sections that were incubated with normal rabbit serum prior to the fluorochroming procedure. These values are of essentially the same magnitude as those in Table II, thus showing that there was no detectable inhibition of the specific fluorescence by normal rabbit serum. In contrast, the average glomerular-tubular ratio of fluorescence intensity was reduced to 1.0, and

the confidence interval to 1.0 to 1.1, in sections that were incubated with uncoupled rabbit antibodies against human globulins prior to the fluorochroming procedure. These values obtained after inhibition of the specific fluorescence are essentially of the same magnitude as those observed in the control group of kidneys (Table I) without any attempts at inhibiting the fluorescence. Text-figure 2 is illustrative in




Text-figure 2. Glomerular-tubular ratio of intensity of characteristic fluorescence, plotted on the horizontal axis, for fluor-treated sections of human kidneys in (a) the glomerulonephritis group, when the sections are incubated with normal rabbit serum prior to the fluorochroming procedure, and in (b) the glomerulonephritis group, when the sections are incubated with uncoupled rabbit autibodies against human gamma globulins prior to the fluorochroming procedure. The specific fluorescence is blocked, and curve b in this figure looks very similar to curve a, for the controls, in Text-figure 1.

this respect when compared with Text-figure 1. Notable is the similarity in the curves on the right half of each figure, the one, Text-figure 1 (b), being obtained from the analysis of sections of the glomerulonephritis group treated with the standard fluorochroming procedure and the other, Text-figure 2 (a), from similar sections incubated with normal rabbit serum prior to the fluorochroming procedure.

The curves on the left half of each figure are likewise similar: the one, Text-figure 1 (a), being obtained from the analysis of sections of the control kidneys treated with the standard fluorochroming procedure and the other, Text-figure 2 (b), from sections of the glomerulonephritis group incubated with uncoupled rabbit antibodies against human globulins prior to the fluorochroming procedure.

Further evidence for the immunologic specificity of the standard fluorochroming procedure was obtained, as described previously, by the specific removal of antibody from aliquots of the fluor by precipitation with human $\gamma_2$-globulin. These aliquots, now lacking fluorescein-labeled antibodies against human gamma globulins, no longer imparted characteristic fluorescence to the glomerular lesions. For example, after their use on sections from the case of lipid nephrosis, the average glomerular-tubular ratio of fluorescence intensity was found to be 0.8 and the 99 per cent confidence interval, 0.77 to 0.83, for 21 determinations of the ratio. Ancillary data were obtained by the use of a preparation of fluorescein-labeled antibodies against *rat* globulins. The average glomerular-tubular ratio of fluorescence intensity was then found to be 0.7 and the 99 per cent confidence intervals, 0.4 to 1.1, for 13 determinations of the ratio in sections from the case of lipid nephrosis.

The quantitative data, therefore, in all respects fully support the conclusions obtained by visual inspection of the fluor-treated sections in the fluorescence microscope. Gamma globulins are specifically localized in glomeruli in lipid nephrosis of childhood and in glomerulonephritis in all ages.

### *Periarteritis Nodosa*

The evidence for the glomerular localization of gamma globulins in acute glomerulonephritis associated with periarteritis nodosa has been given in the preceding section. As shown in Table II, the average glomerular-tubular ratio of fluorescence intensity was 2.6 in this disease, just as was the average for all types of glomerulonephritis studied.

The characteristic fluorescence imparted by the fluor also was observed in the arterial lesions in periarteritis nodosa (Fig. 24). The specific yellow-green fluorescence, signifying localization of globulins and occurring in the intima and the media at sites of necrotizing angiitis, was readily distinguished from the intrinsic blue-white fluorescence of elastic and collagenous fibers in the arterial wall. For quantitative purposes the specific fluorescence intensity of the arterial lesions in periarteritis nodosa was compared with the renal tubular intensity in the immediate neighborhood of the blood vessel. The aver-

age ratio (Table IV) was 2.8 in sections incubated with normal serum before being treated with the fluor; whereas, prior treatment of sections with uncoupled rabbit antibodies against human globulins effectively inhibited the specific fluorescence, reducing the average ratio to 1.4. Thus, although the observations are limited in number and were made upon a single case, the evidence indicates that gamma globulins are localized in the acute vascular lesions of periarteritis nodosa in man, whether occurring in the arteries or in the glomerular capillary tufts.

### *Renal Amyloidosis*

The characteristic fluorescence imparted by the fluor occurred in the glomeruli, notably in the thickened glomerular capillary walls, in renal amyloidosis (Fig. 25). Since in this case glomeruli with characteristic fluorescence, signifying globulin localization, were much more numerous than those with deposits of amyloid (as revealed by standard staining reactions for amyloid), it appears that globulins are localized before amyloid deposition is microscopically detectable. To say more than this is not justified by the evidence at hand.

The prior treatment of sections with uncoupled rabbit antibodies

TABLE IV

*Specific Fluorescence Intensity of Arterial Lesions, Relative to Renal Tubular Intensity, in Sections of Human Kidney (Periarteritis Nodosa) Treated with the Fluorochroming Procedure for Demonstration of Localizing Human Gamma Globulins, and Inhibition of Specific Fluorescence by Prior Treatment with Uncoupled Rabbit Antibodies Against Human Globulins*

| Section treatment | Ratio of fluorescence intensity | | |
|---|---|---|---|
| | Av. | Ci. | N. |
| Normal rabbit serum | 2.8 | 2.0–3.6 | 5 |
| Uncoupled rabbit antibodies | 1.4 | 1.2–1.6 | 4 |

Av. = average.
Ci. = confidence interval which includes 99 per cent of the estimates of the average.
N. = number of measurements.

against human globulins completely inhibited the fluorochroming reaction in renal amyloidosis (Fig. 26). After the specific removal of antibody from aliquots of the fluor by precipitation with human $\gamma_2$-globulin, the picture was identical with that in Figure 26. These aliquots, lacking fluorescein-labeled antibodies against human gamma globulins, no longer imparted characteristic fluorescence to the glomeruli.

Quantitative data are given in Table V. The average glomerular-tubular ratio of fluorescence intensity in sections treated with the standard fluor was 2.5, very similar in magnitude to the average for

the glomerulonephritis group. Prior treatment of sections with uncoupled rabbit antibodies against human globulins reduced the average ratio to 1.0.

Thus, although the observations are limited to a single case, the evidence indicates that gamma globulins are localized in the glomeruli, notably in the thickened glomerular capillary walls, in renal amyloidosis.

TABLE V

*Glomerular-Tubular Ratio of Fluorescence Intensity in Sections of Human Kidney (Renal Amyloidosis) Treated with the Fluorochroming Procedure for Demonstration of Localizing Human Gamma Globulins; and Inhibition of Specific Fluorescence by Prior Treatment with Uncoupled Rabbit Antibodies Against Human Globulins*

| Section treatment | Ratio of fluorescence intensity | | |
|---|---|---|---|
| | Av. | Ci. | N. |
| Standard fluorochroming procedure | 2.5 | 2.3–2.8 | 33 |
| Normal rabbit serum | 2.3 | 1.9–2.8 | 22 |
| Uncoupled rabbit antibodies | 1.0 | 0.9–1.0 | 26 |

Av. = average.
Ci. = confidence interval which includes 99 per cent of the estimates of the average.
N. = number of measurements.

## DISCUSSION

The belief that glomerulonephritis in man is of allergic origin has been expressed by clinicians, among them von Pirquet and Schick[5] and Volhard,[6] during the last half century. The evidence, much of which was cited by Fishberg[14] in 1939, in favor of the view that immuno-allergic mechanisms are in some way connected with the development of glomerulonephritis includes (a) the observation that glomerulonephritis complicating scarlatina or tonsillitis occurs during convalescence rather than at the height of the infection; (b) the not uncommon association of clinical glomerulonephritis with serum sickness; (c) the increased skin reactivity to filtrates of cultures of hemolytic streptococci shown by patients with glomerulonephritis; (d) an elevated titer of antibodies, including anti-streptolysin, in the blood of patients with glomerulonephritis following scarlatina or pharyngitis; (e) the more frequent occurrence of glomerulonephritis in subacute bacterial endocarditis during the bacteria-free rather than the bacteremic stage of the disease; (f) a decreased titer of serum complement in acute glomerulonephritis; and (g) the experimental production of glomerulonephritis in animals by immuno-allergic procedures.

The principal contribution of the present work is the demonstration that gamma globulins are localized in the glomerular lesions in glomer-

ulonephritis of man and in so-called lipid nephrosis of childhood. By proving that proteins of the type known to include antibodies are localized at the sites of the lesion, this work fulfills a requirement essential for the immuno-allergic pathogenesis of these diseases. Our investigations of two forms of experimental glomerulonephritis in animals similarly demonstrated the presence of glomerular localizing globulins. In one form of experimental glomerulonephritis,[2] that produced by foreign protein injection, we presumed that the localized globulins were, at least in part, antibodies against some of the injected protein which had circulated through, and become fixed in, the glomeruli. In the other experimental condition,[4] that of nephrotoxic glomerulonephritis, both the nephrotoxic antigen and the localized globulins were shown to occupy similar or identical glomerular sites so that the latter most likely were, at least in part, antibodies against the former. Therefore, in human glomerulonephritis and so-called lipid nephrosis it is reasonable to think that the localized gamma globulins are, at least in part, antibodies against antigens that have localized in the glomeruli. While the antigens conceivably have a variety of bacterial, rickettsial, or other extrinsic origins, it is most logical to presume from the opening discussion that they are of streptococcal origin in some cases. If this is true, it may be possible in further work on this, or similar, pathologic material to demonstrate the presence of glomerular-localizing streptococcal antigens by treating sections with fluorescein-labeled rabbit antibodies against streptococcal antigens. Only after an exhaustive search for a variety of extrinsic antigens has failed, would it seem likely to us that the antibodies are in reality auto-antibodies, acting solely against an intrinsic tissue antigen.

Since commonly the glomerulonephritis initiated in pre-eclampsia and eclampsia of pregnancy is histologically similar to the examples of acute diffuse (membranous) glomerulonephritis studied in the present work, it is reasonable to expect that gamma globulins will be found to be localized in the glomerular lesions in this disease as well.

The immuno-allergic aspects of periarteritis nodosa and other diseases attributed to hypersensitivity have been discussed recently by Rich,[15] and the pertinent historical contributions made during the last century or so are summarized in his Harvey Lecture. Here again, although the observations are limited to but one case, the present work indicates that gamma globulins are localized in the acute necrotizing arterial lesions in periarteritis nodosa, as well as in the glomerular lesions in acute glomerulonephritis associated with this disease. A variety of antigens could, by their vascular localization, give rise to similarly localizing antibodies. Preliminary observations of necrotiz-

ing allergic angiitis induced in rabbits by the injection of purified foreign proteins had previously suggested to us that rabbit globulins were localized in the sites of the lesions.[2]

The knowledge that hyperimmune animals, particularly horses, used in the commercial production of antisera, frequently develop amyloidosis has led to the belief that immuno-allergic mechanisms are involved in the origin of amyloid. The present investigation demonstrates that gamma globulins are localized in the glomerular capillary walls in renal amyloidosis at a stage which precedes the deposition of microscopically detectable amyloid.

Our investigations, then, have disclosed a feature common to such diverse diseases as lipid nephrosis and glomerulonephritis, periarteritis nodosa, and parenchymatous amyloidosis, namely, the localization of gamma globulins in the sites of active lesions. This finding provides the first direct evidence for the immuno-allergic pathogenesis of these diseases. It seems reasonable to expect that the pathogenesis of glomerulonephritis initiated in eclampsia of pregnancy, of other so-called collagen diseases in addition to periarteritis nodosa (notably rheumatic fever and rheumatoid arthritis), of certain hemolytic anemias and purpuras, and of classical allergic diseases themselves (such as bronchial asthma and anaphylactic shock), will be further elucidated by the analytical procedures described in this study. Moreover, the tissue responses in certain diagnostic immunologic reactions, for example, the tuberculin and the Schick tests, may be investigated similarly, as also may be the tissue mechanisms in antibacterial and antitoxic immunity.

The foregoing discussion deals with the tissue localization of gamma globulins and antibodies which we presume have been fabricated elsewhere in the body. The cellular production of immune globulins also can be studied by the methods described. Our preliminary observations suggested[2] and other work has established[16] that plasma cells are most active in this capacity, a conclusion already reached by several investigators[17-21] using different methods of study.

In the field of oncology, the most pressing application of the method appears to be in the study of the controversial rôle of tumor-localizing antibodies in the development of immunity to cancer. In surgery, the factors which inhibit the growth of tissues when transplanted from man to man may be capable of further analysis by this method, first, of course, by recourse to the experimental counterpart in animals.

It may be asked whether the technique may be developed someday to the level of diagnostic usefulness. The lack of commercial availability of the fluorochroming reagents appears to be the main practical

limitation, for all other supplies and equipment are readily obtained by laboratories of pathology and clinical pathology. For the foreseeable future, however, the principal applications appear to be in the investigations of the pathogenesis of several diseases wherein, it is hoped, a better understanding will lead to better means of treatment and prevention.

## Summary and Conclusions

The unique feature of this study is the histopathologic application of a microfluorescence method for demonstrating the histologic sites of localized human gamma globulins.

The principal contribution of the work is to establish beyond a reasonable doubt that gamma globulins are localized in the active glomerular lesions in so-called lipid nephrosis (chronic membranous glomerulonephritis) of childhood and in various types of glomerulonephritis occurring in man at all ages. Evidence is presented also that gamma globulins are localized in the glomerular and the arterial lesions in periarteritis nodosa and in the glomerular lesions in renal amyloidosis.

By demonstrating that proteins of the type known to include antibodies are localized in the sites of the lesions, the observations fulfill a requirement essential for the immuno-allergic pathogenesis of these several diseases of man. It is logical to think, for reasons given in the text, that the localized gamma globulins are, at least in part, antibodies against antigens that have localized in the prospective sites of the lesions.

Success in the present investigation suggests that the pathogenesis of a variety of diseases, among them, glomerulonephritis initiated in eclampsia of pregnancy, rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus and other so-called collagen diseases, certain hemolytic anemias and purpuras, as well as classical allergic diseases themselves, such as bronchial asthma, will be further elucidated by the analytical procedure used in this study.

We wish to thank Dr. Wilbur E. Noyes, Dr. Leonhard Korngold, Mr. John Hlinka, and Miss Betty Corcoran for their assistance.

## REFERENCES

1. Mellors, R. C.; Siegel, M., and Pressman, D. Analytical pathology. I. Histochemical demonstration of antibody localization in tissues, with special reference to the antigenic components of kidney and lung. *Lab. Investigation,* 1955, 4, 69–89.
2. Mellors, R. C.; Arias-Stella, J.; Siegel, M., and Pressman, D. Analytical pathology. II. Histopathologic demonstration of glomerular-localizing antibodies in experimental glomerulonephritis. *Am. J. Path.,* 1955, 31, 687–715.

3. Mellors, R. C. Histochemical demonstration of the *in vivo* localization of antibodies: antigenic components of the kidney and the pathogenesis of glomerulonephritis. *J. Histochem. & Cytochem.*, 1955, **3**, 284–289.

4. Ortega, L. G., and Mellors, R. C. In preparation.

5. von Pirquet, C., and Schick, B. Die Serumkrankheiten. Franz Deuticke, Leipzig & Wien, 1905, pp. 60–68.

6. Volhard, F. Die doppelseitigen hämatogenen Nierenerkrankungen (Bright'sche Krankheit). Julius Springer, Berlin, 1918, pp. 358–472.

7. Coons, A. H., and Kaplan, M. H. Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody. *J. Exper. Med.*, 1950, **91**, 1–13.

8. Deutsch, H. F. Separation of antibody-active proteins from various animal sera by ethanol fractionation techniques. In: Methods in Medical Research, Corcoran, A. C. (ed.). Year Book Publishers, Inc., Chicago, 1952, **5**, 284–300.

9. Ouchterlony, O. Antigen-antibody reactions in gels. IV. Types of reactions in coordinated systems of diffusion. *Acta path. et microbiol. Scandinav.*, 1953, **32**, 231–240.

10. Korngold, L., and Lipari, R. Tissue antigens of human tumors grown in rats, hamsters, and eggs. *Cancer Research*, 1955, **15**, 159–161.

11. Bush, V., and Hewitt, R. E. Frozen sectioning. A new and rapid method. *Am. J. Path.*, 1952, **28**, 863–873.

12. Bell, E. T. Renal Diseases. Lea & Febiger, Philadelphia, 1946, 434 pp.

13. Allen, A. C. The Kidney. Grune & Stratton, New York, 1951, 583 pp.

14. Fishberg, A. M. Hypertension and Nephritis. Lea & Febiger, Philadelphia, 1939, ed. 4, p. 448.

15. Rich, A. R. Hypersensitivity in disease with especial reference to periarteritis nodosa, rheumatic fever, disseminated lupus erythematosus and rheumatoid arthritis. *Harvey Lect.*, 1946–47, **42**, 106–147.

16. Coons, A. H.; Leduc, E. H., and Connolly, J. M. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. Exper. Med.*, 1955, **102**, 49–60.

17. Kolouch, F., Jr. Origin of bone marrow plasma cell associated with allergic and immune states in the rabbit. *Proc. Soc. Exper. Biol. & Med.*, 1938, **39**, 147–148.

18. Bjørneboe, M., and Gormsen, H. Experimental studies on the rôle of plasma cells as antibody producers. *Acta path. et microbiol. Scandinav.*, 1943, **20**, 649–692.

19. Bing, J.; Fagraeus, A., and Thorell, B. Studies on nucleic acid metabolism in plasma cells. *Acta physiol. Scandinav.*, 1945, **10**, 282–294.

20. Fagraeus, A. The plasma cellular reaction and its relation to the formation of antibodies *in vitro*. *J. Immunol.*, 1948, **58**, 1–13.

21. Moeschlin, S.; Pelaez, J. R., and Hugentobler, F. Experimental investigations of the relationship between plasma cells and antibody formation (phase contrast microscope). *Acta haemat.*, 1951, **6**, 321–334.

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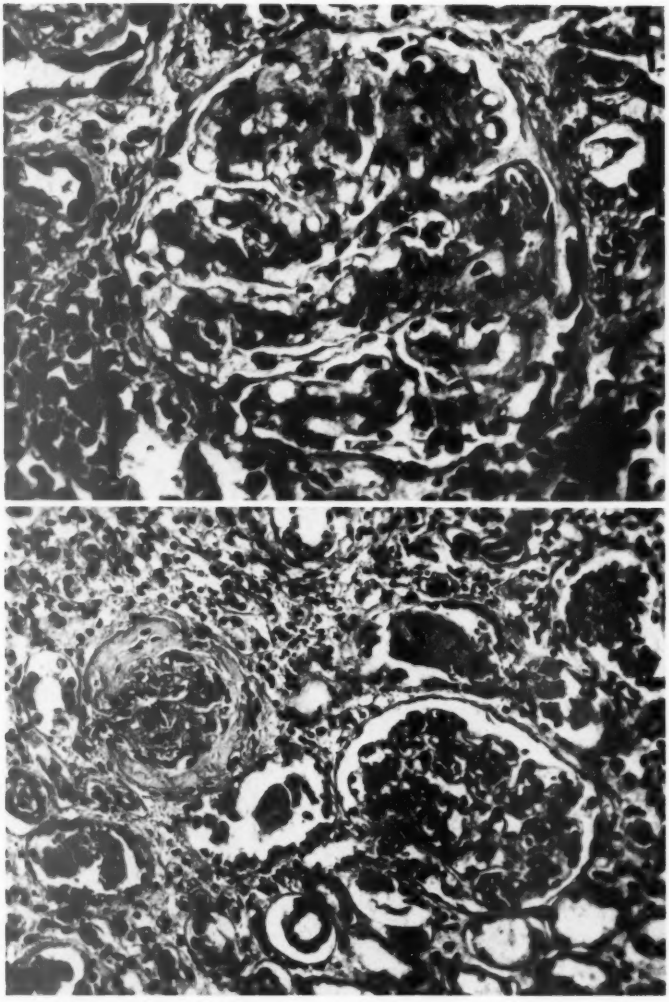
[ *Illustrations follow* ]

## LEGENDS FOR FIGURES

FIGS. 1 to 7. Paraffin sections stained with hematoxylin and eosin.

FIG. 1. Thickening of the glomerular capillary walls producing partial occlusion of some of the capillary lumina in lipid nephrosis (chronic membranous glomerulonephritis) in childhood. The characteristic glomerular fluorescence, indicating sites of localization of gamma globulins in this case, is seen in Figures 13 to 15.

FIG. 2. Increase in endothelial cellularity together with some thickening of the capillary walls in one glomerulus, fibrosis in another, and tubular protein casts in chronic glomerulonephritis (chronic sclerosing and membranous glomerulonephritis) in childhood accompanied by the nephrotic syndrome. The specific glomerular fluorescence, indicating sites of localization of gamma globulins in this case, is seen in Figure 18.



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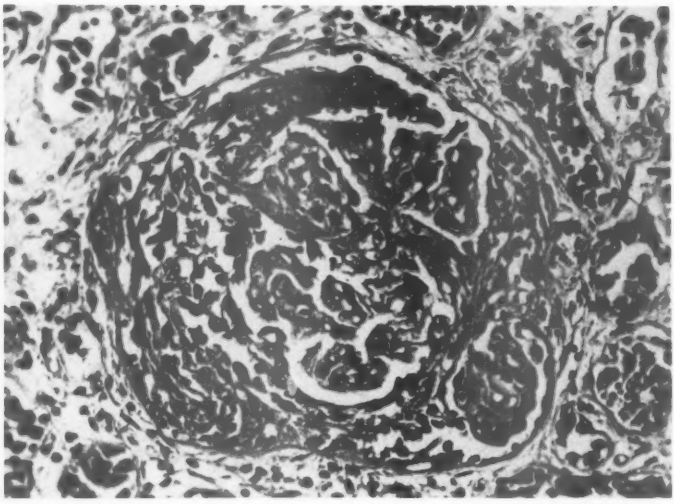
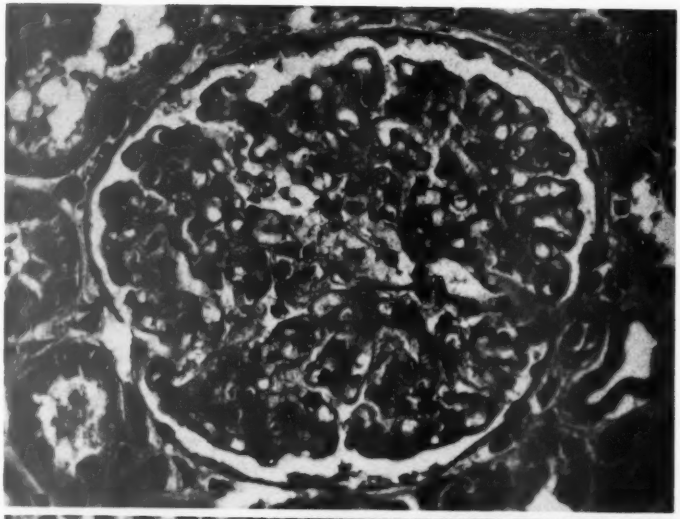


FIG. 3. Extravasation of red blood cells into Bowman's space and into the appertaining tubular lumina, cellular and fibrous crescents, and glomerular fibrosis in combined, acute, and subacute diffuse glomerulonephritis in an adult female. The specific glomerular fluorescence, indicating sites of localization of gamma globulins in this case, is given in Figure 19.

FIG. 4. Thickening of the glomerular capillary walls and enlargement of the glomerulus in acute diffuse membranous glomerulonephritis in an adult female. The specific glomerular fluorescence, indicating site of localization of gamma globulins in this case, is illustrated in Figure 20.

FIG. 5. Fibrinoid necrosis and acute exudative inflammation, destroying a portion of the wall of a small artery and extending into the periadvential tissue, together with early organization of the luminal contents in periarteritis nodosa of the kidney. The specific fluorescence, indicating arterial sites of localization of gamma globulins in this case, is seen in Figure 24.



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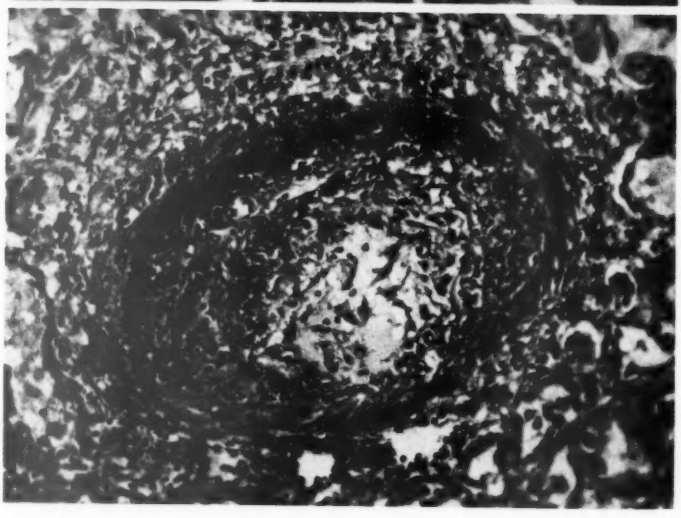

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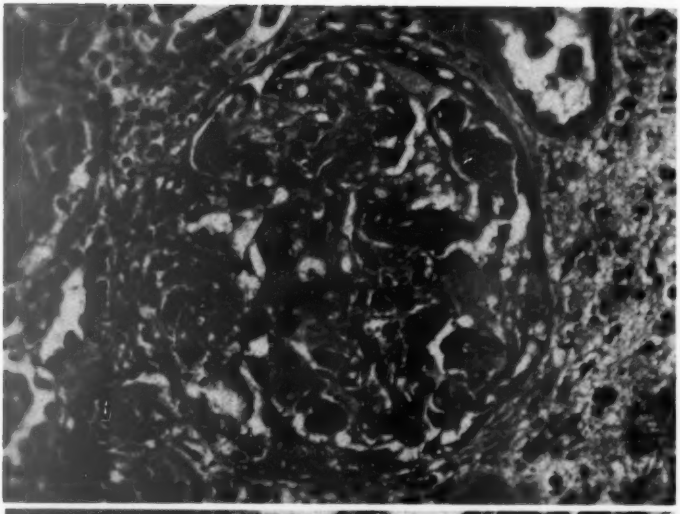
FIG. 6. Fibrinoid necrosis, acute exudative inflammation, and complete disorganization of glomerular architecture in necrotizing glomerulonephritis associated with periarteritis nodosa. The specific fluorescence, indicating glomerular sites of localization of gamma globulins in this case, is illustrated in Figure 23.

FIG. 7. Thickening of the glomerular capillary walls, focal deposition of amyloid, and tubular protein casts in renal amyloidosis. The specific glomerular fluorescence, indicating site of localization of gamma globulins in this case, is seen in Figure 25.



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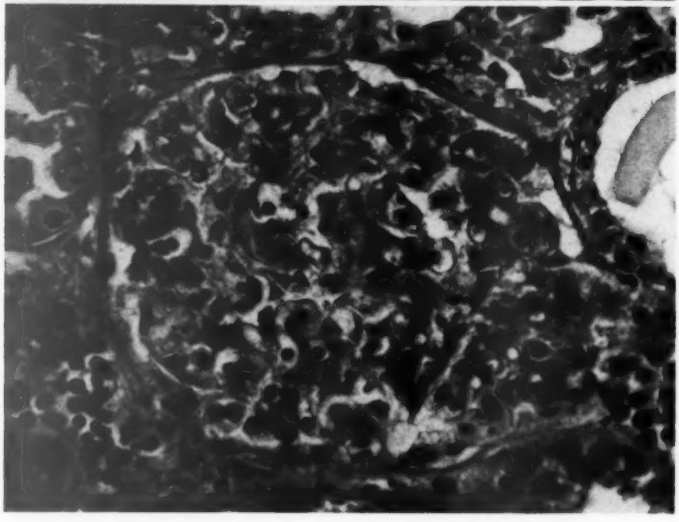

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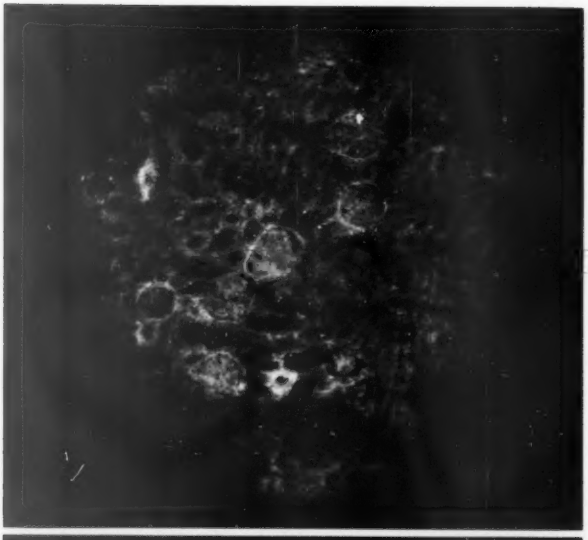
FIGS. 8 to 26. Fluorescence photomicrographs of unfixed, frozen sections treated, except where otherwise stated, with the fluorochroming procedure for demonstrating sites of localization of gamma globulins in human tissues by imparting a characteristic (yellow-green) fluorescence.

FIG. 8. Essentially normal, control kidney from a child in the first decade of life. The intensity of the characteristic fluorescence is nearly the same in the glomeruli and in the tubules. There is no specific localization of gamma globulins.

FIG. 9. Control kidney from an adult in the fifth decade of life with acute suppurative pyelonephritis. The intensity of the characteristic fluorescence is nearly the same in the glomeruli and in the tubules. There is no specific localization of gamma globulins. An occasional particle in the tubules displays intrinsic fluorescence, which, under the microscope, is readily differentiated by its color from the characteristic fluorescence that signifies localizing gamma globulins.



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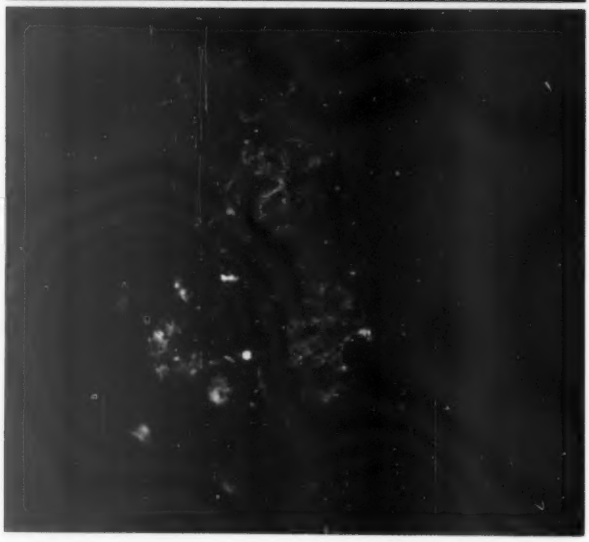

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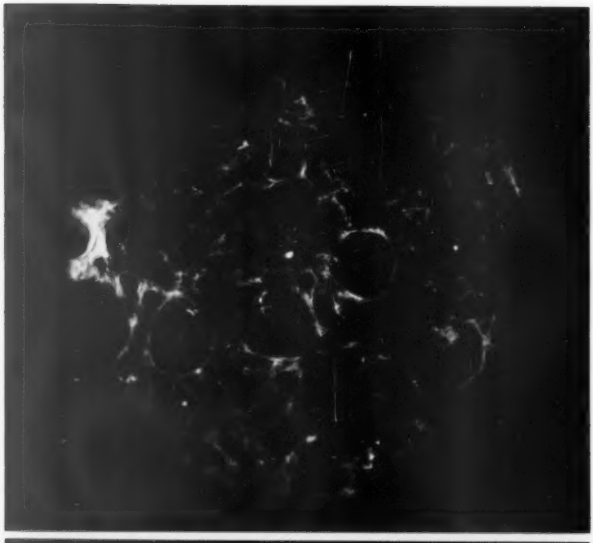
FIG. 10. Control kidney from an adult in the sixth decade of life with acute suppurative pyelonephritis. The descriptive comments for Figure 9 pertain here as well. There is no specific localization of gamma globulins. The intrinsic fluorescence of interstitial connective tissue surrounding some of the glomerular capsules and of connective tissue in a small artery may be seen also.

FIG. 11. Control kidney from an adult in the seventh decade of life with arterial and arteriolar nephrosclerosis. The descriptive comments for Figures 9 and 10 pertain here as well. There is no specific localization of antibodies.



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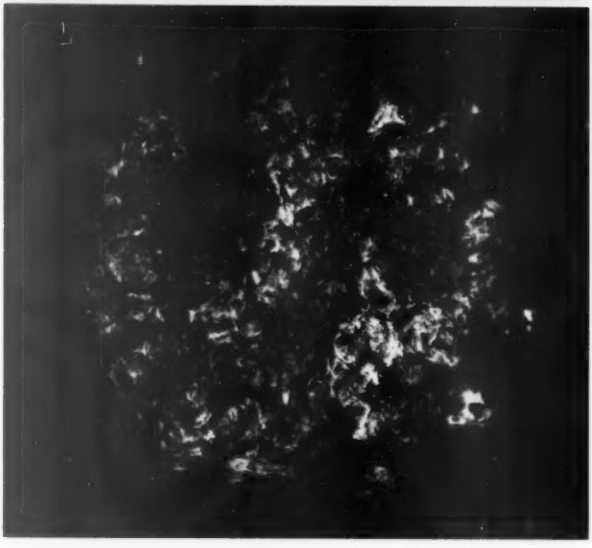

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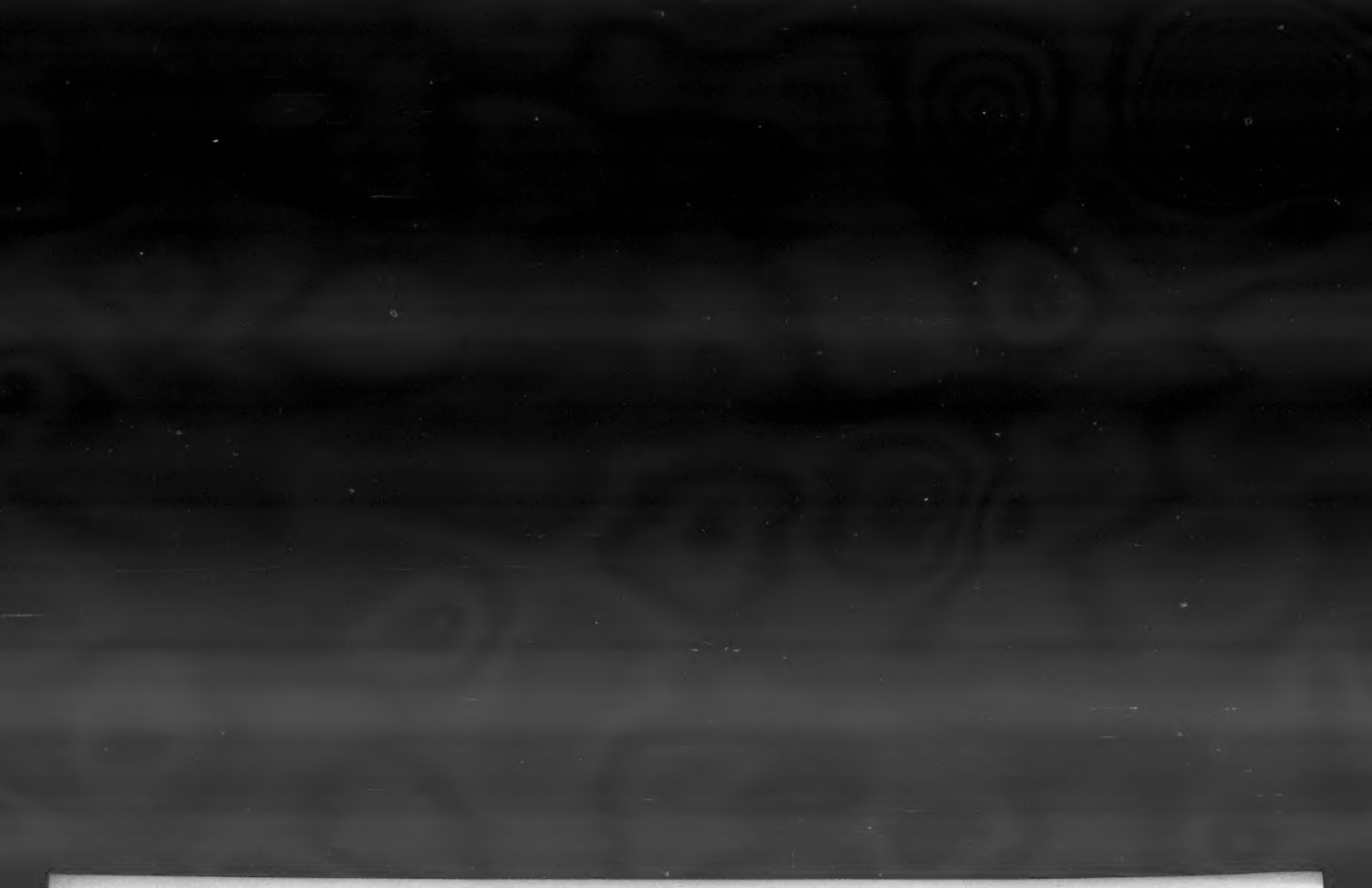
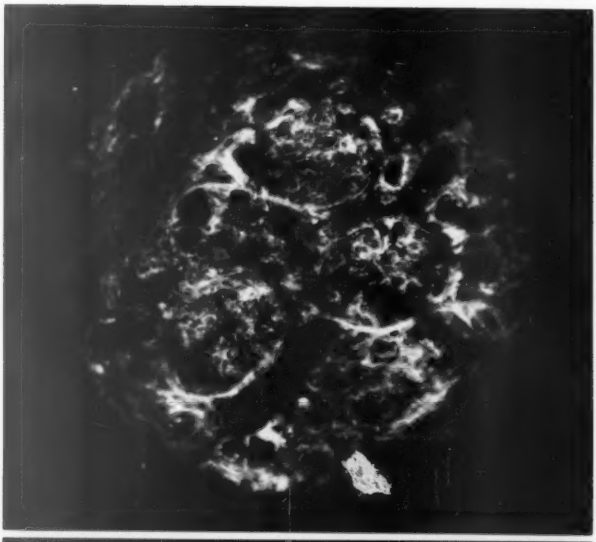
FIG. 12. Control kidney from an adult in the eighth decade of life with arterial nephrosclerosis. The descriptive comments for Figures 9 and 10 pertain here as well. There is no specific localization of antibodies.

FIG. 13. Lipid nephrosis (chronic membranous glomerulonephritis) in childhood (see also Fig. 1), with characteristic glomerular fluorescence indicating localization of gamma globulins in the glomerular tuft. Lipid and other particles in the tubules display intrinsic fluorescence, which, under the microscope, is readily distinguished by its color from the characteristic fluorescence that signifies localizing gamma globulins.





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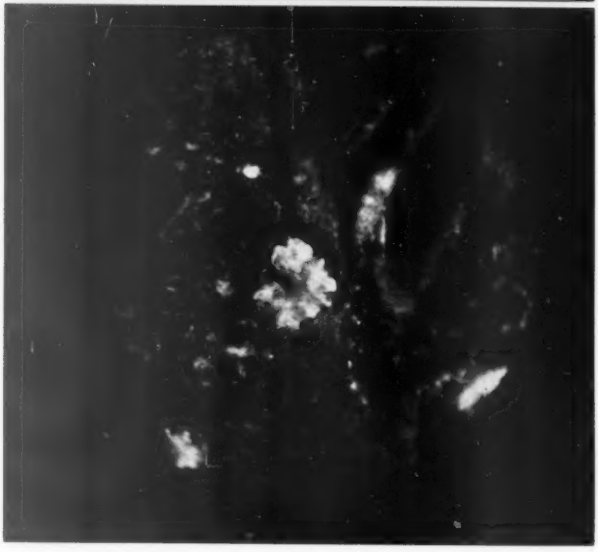

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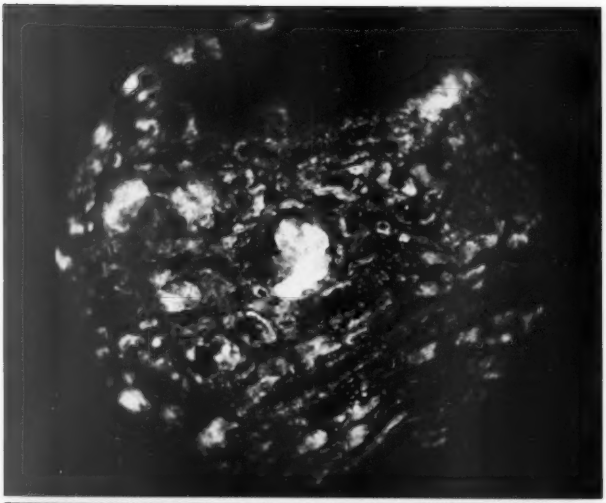
FIG. 14. Same case of lipid nephrosis as that from which Figure 13 was taken, with the descriptive comments for Figure 13 applying here as well. The gamma globulins are localized in a portion of the glomerulus.

FIG. 15. Same case of lipid nephrosis, as that from which Figures 13 and 14 were taken. The characteristic fluorescence signifying the localization of gamma globulins is seen in portions of two glomeruli, that in the upper right glomerulus occurring in what appears dimensionally to be coiled capillaries. The section was incubated with normal rabbit serum prior to the fluorochroming procedure.



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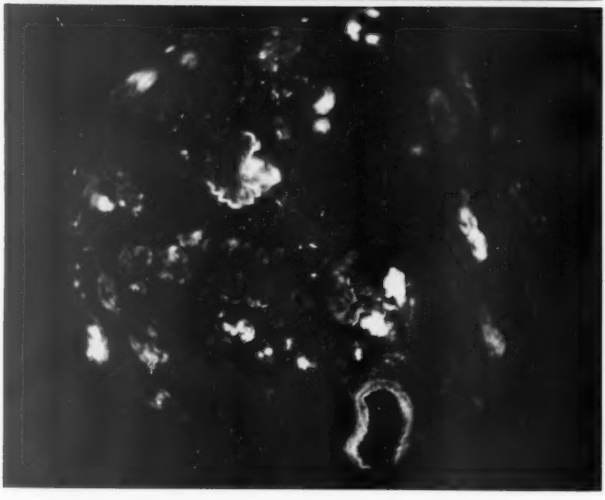

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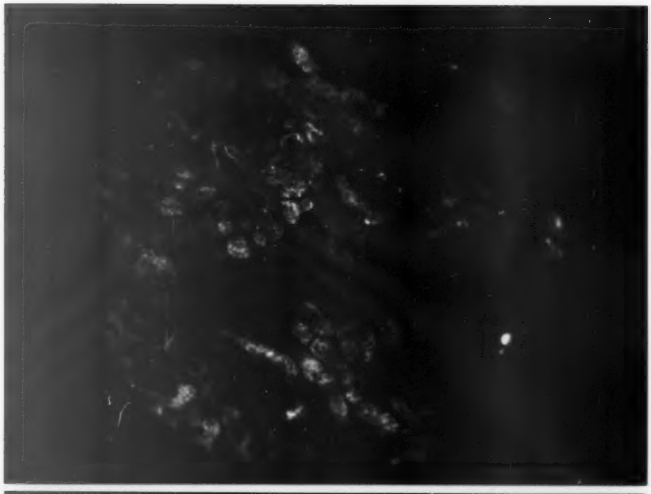
FIG. 16. Same case of lipid nephrosis as that from which Figures 13 to 15 were taken, but here the characteristic fluorescence has been blocked by incubating the section with uncoupled rabbit antibodies against human gamma globulins prior to the fluorochroming procedure. The fluorescence of the centrally located glomerulus can now scarcely be perceived. The intrinsic and the non-specific fluorescence of the tubules, of a blood vessel, and of other histologic structures persists undiminished. A similar picture occurred in this case when sections were treated with aliquots of the standard fluor from which specific antibodies had been removed by precipitation with pure human $\gamma_2$-globulin. Such aliquots, lacking fluorescein-labeled rabbit antibodies against human gamma globulins, no longer imparted characteristic fluorescence to the glomeruli.

FIG. 17. Same case of lipid nephrosis as that from which Figures 13 to 16 were taken. However, the section was incubated with a preparation of fluorescein-labeled rabbit antibodies against *rat* globulins (not against *human* globulins, as in the standard fluorochroming procedure). No characteristic fluorescence is imparted to the two glomeruli in the field.



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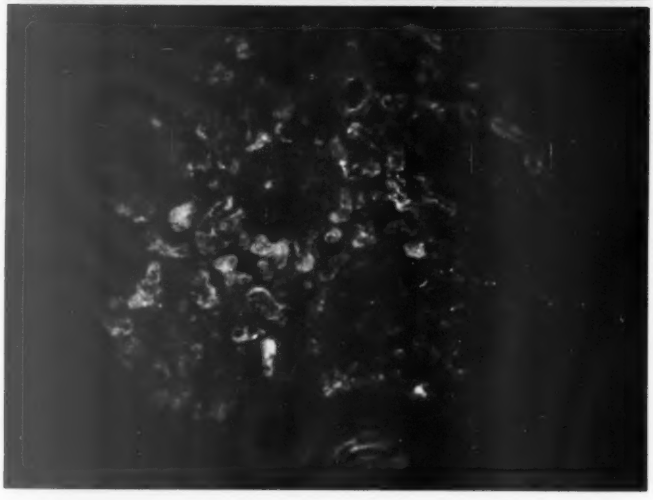

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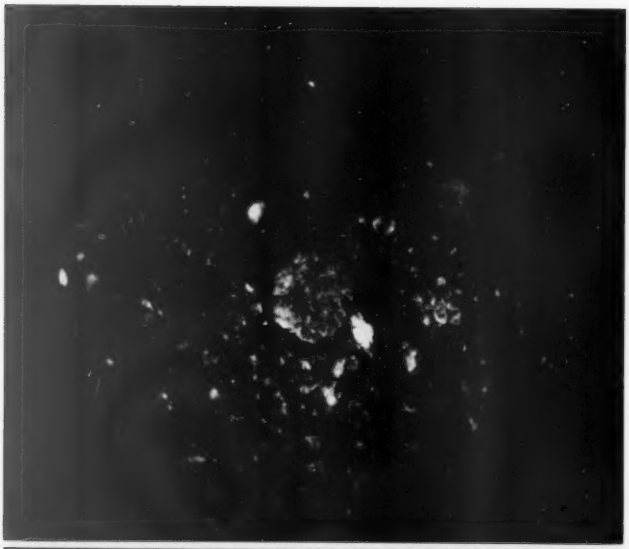
FIG. 18. Chronic glomerulonephritis (chronic membranous and sclerosing glomerulonephritis) in childhood, with the nephrotic syndrome (see also Fig. 2). The characteristic fluorescence, indicating localization of gamma globulins, occurs in glomeruli, such as that illustrated, which have not undergone fibrosis. Lipid and other particles, abundant in the tubules, display intrinsic fluorescence.

FIG. 19. Combined acute and subacute diffuse glomerulonephritis in an adult (see also Fig. 3), with characteristic fluorescence indicating localization of gamma globulins in the glomerular tuft but not in the glomerular crescent.



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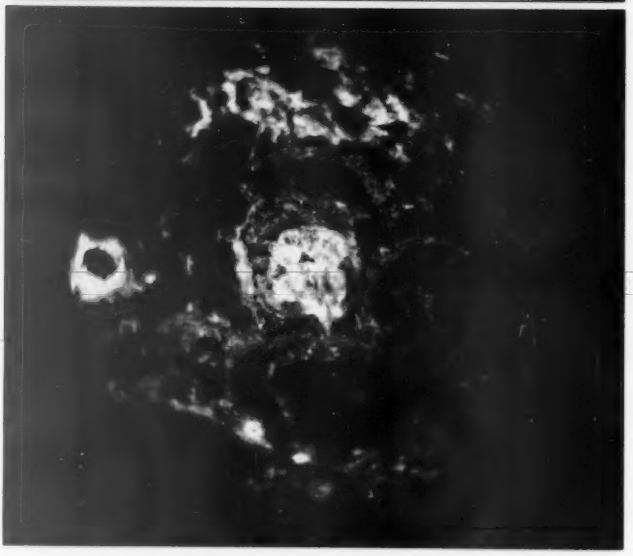

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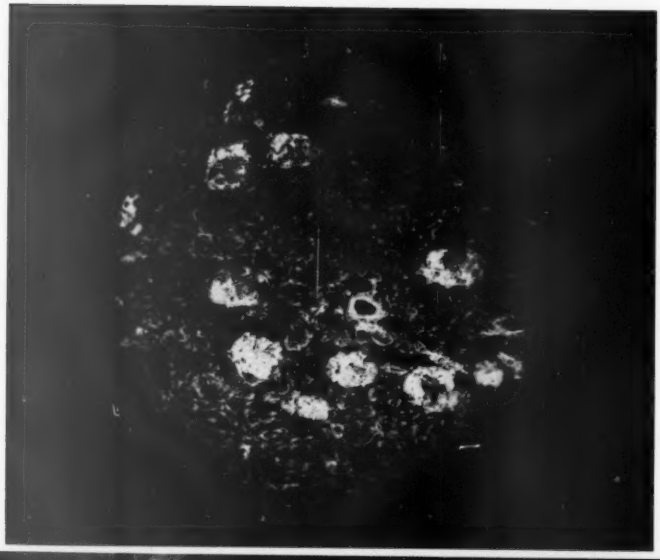
FIG. 20. Acute diffuse membranous glomerulonephritis in an adult (see also Fig. 4), with characteristic fluorescence indicating localization of gamma globulins in the glomeruli. The section was incubated with normal rabbit serum prior to the fluorochroming procedure.

FIG. 21. Acute diffuse membranous glomerulonephritis in an adult, with characteristic fluorescence indicating localization of gamma globulins in the glomerular capillary walls.



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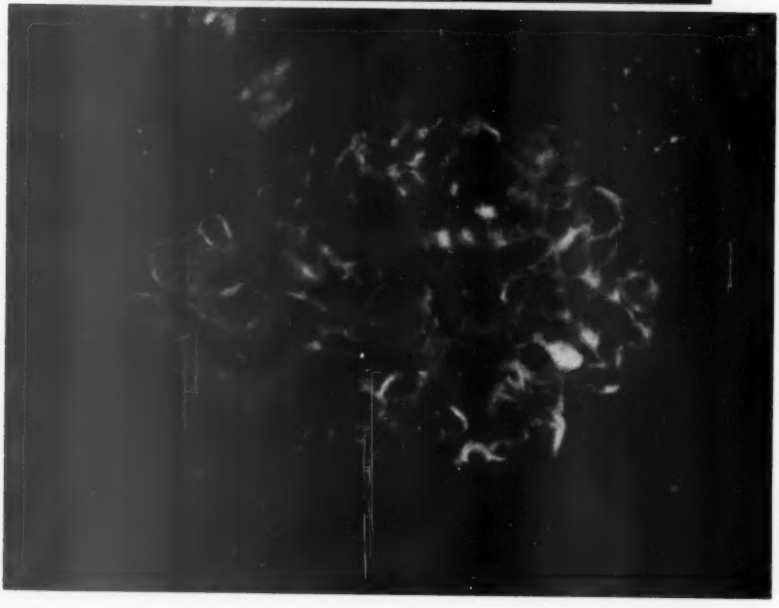

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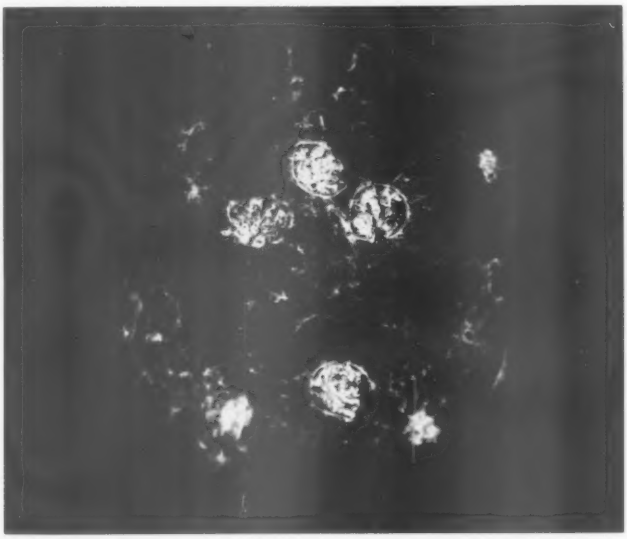


FIG. 22. Acute diffuse membranous glomerulonephritis in an adult, with characteristic fluorescence indicating localization of gamma globulins in the glomeruli.

FIG. 23. Acute necrotizing glomerulonephritis associated with periarteritis nodosa in an adult (see also Fig. 6), with characteristic fluorescence indicating localization of gamma globulins in a glomerulus.

FIG. 24. Periarteritis nodosa (see also Fig. 5), with characteristic fluorescence indicating localization of gamma globulins in a segment of the arterial wall, specifically in the intima and internal to the elastic lamellae. The section was incubated with normal rabbit serum prior to the fluorochroming procedure.

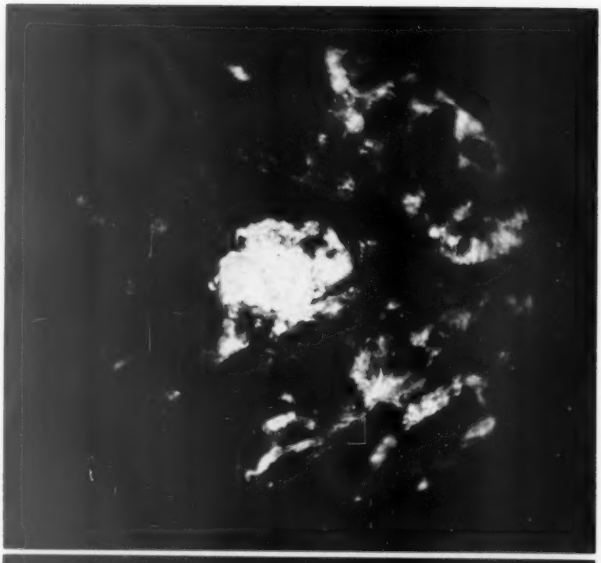

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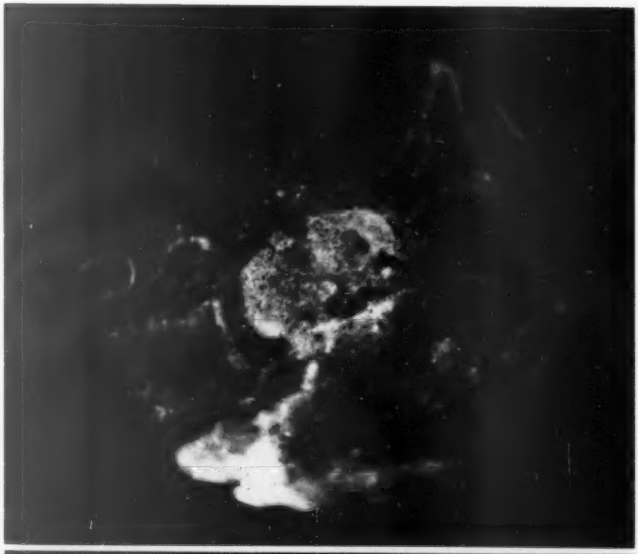
FIG. 25. Renal amyloidosis (see also Fig. 7), with characteristic fluorescence indicating localization of gamma globulins in the glomerulus, notably in the glomerular capillary walls.

FIG. 26. Same case of renal amyloidosis as that from which Figure 25 was taken, but here the characteristic fluorescence has been blocked by incubating the section with uncoupled rabbit antibodies against human gamma globulins prior to the fluorochroming procedure. A similar picture occurred in this case when sections were treated with aliquots of the standard fluor from which specific antibodies had been removed by precipitation with pure human $\gamma_2$-globulin. Such aliquots, lacking fluorescein-labeled rabbit antibodies against human gamma globulins, no longer imparted characteristic fluorescence to the glomeruli.



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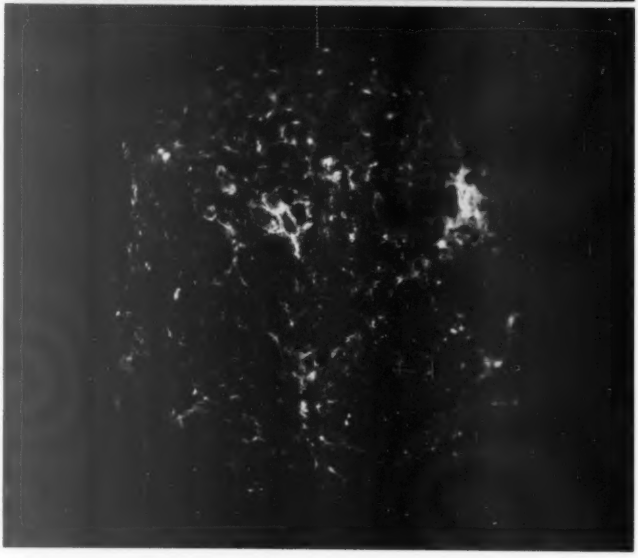

26

# CARCINOMA OF PARANASAL SINUSES

## Its Histogenesis and Classification *

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The purpose of this paper is to present the histogenesis of carcinomas of the paranasal sinuses, and a classification of them based on this genesis. As in pulmonary carcinomas, carcinoma of the paranasal sinus often shows diverse structure in different areas, and hence there is little significance in subdividing these carcinomas morphologically. More attention should be paid to the principles and limits underlying the structural variations as being more important and basic. As far as I am aware, there have been no investigations along these lines, although numerous reports have appeared in recent years on carcinomas of the paranasal sinuses.[1–6] Ringertz[7] presented a review of the histopathologic and genetic aspects of carcinomas of the nasal and paranasal cavities and, on the basis of numerous cases examined, he made a histologic classification, accompanied by anatomicopathologic descriptions. His paper may be considered the only thorough report thus far, but the part regarding the histogenesis and polymorphic nature of these tumors, especially of the types of salivary gland tumor and paranasal glandular tumors, is not sufficiently clear. The present paper, therefore, attempts further clarification of these matters.

### Materials and Method

As material, 72 cases of carcinoma originating from the mucous membrane of the maxillary and ethmoidal sinuses were used. Those of obscure origin and involving the nasopharynx, alveolar process, and hard palate were excluded. In general, tissue specimens were obtained surgically, but 7 were from necropsies. Classification based on outstanding morphologic features was as follows:

| | |
|---|---|
| Squamous cell carcinoma | 41 cases |
| Basal cell carcinoma | 9 cases |
| Small round cell carcinoma | 2 cases |
| Columnar cell carcinoma | 3 cases |
| Clear cell carcinoma | 2 cases |
| Adenocarcinoma (divided into adenoid cystic, myxo- | |

* Received for publication, June 21, 1955.

| | |
|---|---|
| fibrous, mucous cell, clear cell, solid or trabecular, and acanthomatous subtypes) | 8 cases |
| Unclassified carcinoma | 7 cases |
| Total | 72 cases |

In addition, 4 papillomas and 2 adenomas served as contrast materials for precancerous states, and 125 samples of paranasal mucosa from normal and empyemic patients served for studies on squamous metaplasia. Also, for histologic comparison, cancers of the epithelium of the nasal cavity, nasopharynx, and bronchus, together with salivary gland tumors, were examined. The ages of those investigated varied from 32 years (a patient 5 years old was excluded) to 74 years, with an average of 53 years. The highest frequency distribution was seen in the age group 40 to 59 years, and accounted for 64 per cent of all cases. About 63 per cent were males. Sections were prepared from various parts of the tumor and the histologic characteristics were studied microscopically. All specimens were fixed in formalin and stained by ordinary methods. In addition, histochemical stains for mucin and glycogen were made as needed.

## Observations and Results

### *The Paranasal Mucous Membrane of Normal Subjects*

The mother tissues of the tumors arising from the paranasal mucosa can be traced to the three epithelial components: surface epithelium, glands, and excretory ducts.

1. The surface epithelium, namely, the respiratory epithelium, is pseudo-stratified, ciliated, columnar epithelium, between the cells of which goblet cells are richly interspersed. Of the various cells seen, the basal and goblet cells seem to be the more important constituents responsible for the polymorphic nature of the tumors. The basal cells lie in contact with the basement membrane of the columnar epithelium, and are small, coniform cells arranged in a row, with hyperchromatic, round or short, spindle-shaped nuclei. The goblet cells are unicellular glands secreting mucus containing acid mucopolysaccharide. Many theories have been presented regarding the origin of the goblet cells,[8] but from an oncologic viewpoint they appear to be derived from the basal cells.

2. The glands are generally regarded as mixed mucous glands of tubulo-alveolar structure.[9,10] In other words, they are said to be of mixed mucoserous type with the mucous component predominating. The distribution of the mucous, serous, and mucoserous components

varies with the individual gland, and with the part of the gland examined. At any rate, the terminal portions of the gland are composed of mucous, serous, and mucoserous cells. All of the glands possess a basement membrane, but basket or myo-epithelial cells found in the salivary glands and elsewhere are not seen here.

3. The excretory duct, derived by the inward invagination of the surface epithelium into a crypt, is lined for the major part with respiratory epithelium, as in the case of surface epithelium, and gradually forms a transitional part composed of a layer of columnar and cuboidal epithelium as the terminal portion of the gland is approached. The reason why the name transitional part was employed here is that it does not indicate such structural characteristics as are seen in the striated and intercalated ducts of the major salivary glands. Compared with the salivary glands, the glandular and transitional parts show functionally and structurally a lower grade of differentiation, but they can be said to belong to the same matrix tissue when concerned with tumor formation. In the same sense that Schaper and Cohen[11] called the intercalated ducts zones of proliferation, I would like to attribute a similar significance to the transitional part of the duct.

From these histologic findings, it seems rational to divide tumors of the paranasal sinuses according to the matrix tissue from which they are derived, namely, into two divisions: those having origin in the surface epithelium and the major portion of the excretory duct, and those arising from the transitional part of the duct and the gland itself.

### *Squamous Metaplasia*

In order to understand the histologically polymorphic nature of these tumors, a knowledge of the mechanism of embryologic histogenesis and regeneration of mature tissues is necessary. A point of interest here is the problem of squamous metaplasia as the result of regeneration, a method quite comparable to that in the cervix uteri. In the paranasal mucosa examined, the great majority of the regenerative processes resulted in reversion to the normal columnar epithelium, and in only 3 cases was squamous metaplasia seen. In squamous metaplasia, and also in normal regeneration, proliferation of the basal cells plays the main rôle through their metaplastic and normal differentiation. This is seen also in the major part of the excretory duct. Squamous metaplasia is one form of potential plasticity possessed by the basal cells; still there have been no cases in which definite confirmation has been obtained for metaplasia of normal columnar cells into squamous epithelium as often seen in the tumors.

*Diversity of Morphologic Structures of Carcinoma*

As the origin of all carcinomas examined could actually be traced to either of the two epithelial elements, the surface epithelial and the glandular element, I believe that paranasal carcinomas should be classified into the following three categories, roughly in accordance with Geschickter's classification[12] in which carcinomas are divided according to whether they originate from the surface epithelium or the appendages of the mucous membrane.

1. CARCINOMAS ORIGINATING FROM THE SURFACE EPITHELIUM AND THE MAJOR PART OF THE EXCRETORY DUCT

Though relatively rare, papillomas deserve attention as a precancerous form. These may be derived from inflammatory polyps (Figs. 1 and 2), and they may be transformed into papillary carcinomas (Figs. 3 and 4). It is often difficult, as was described by Willis,[18] to make a sharp distinction between benign papillomas and papillary carcinomas. Recurrence after removal is usual, and carcinomatous change may be a common termination. Ringertz[7] stressed that in papilloma of the nasal and paranasal cavities an "inverted" papillary structure is seen histologically. Such a structure calls for attention in that proliferation of the surface epithelium inwardly shows a genetic reduplication of the excretory duct.

In tumors belonging to this first class, the basal cells are responsible mainly for the polymorphic structure, and in the proliferating stage they become small, basophilic spindle cells with short, spindle-shaped, darkly staining nuclei. A characteristic feature of the proliferating portion of the tumor is the preponderance of these small spindle-shaped cells. Depending on the arrangement of the tumor cells, the neoplasms composed solely of these cells present the various types of basal cell carcinoma, including scirrhous carcinoma and oat-cell carcinoma. In addition, there is a type composed of small round cells which can be considered to be anaplastic basal cells. This type proliferates diffusely and in a disorderly manner, and appears at first sight to be lymphosarcoma (Figs. 5 and 6). Small round cell carcinoma was characterized by a high grade of malignancy, and in the 2 cases studied there was noted early generalized metastasis.

The basal cells appear to retrace the process of normal histogenesis and regeneration, and may show differentiating transformation into columnar cells (Figs. 7 and 8), or metaplastic conversion into squamous cells, resulting in a picture of baso-columnar cell carcinoma or of baso-squamous cell carcinoma. Finally, they may form solid columnar cell carcinoma or squamous cell carcinoma. Too, the columnar cells may become squamous cells through metaplasia; in this way carcinoma

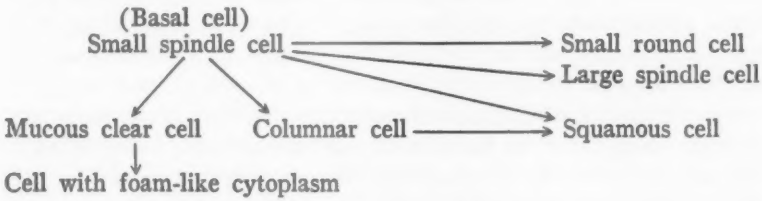
with epithelium in a transitional stage and, further, squamous cell carcinoma are formed. Squamous cell carcinoma is very frequently seen in paranasal carcinomas, and in my material was noted in about 57 per cent of all cases examined. However, from the fact that squamous metaplasia following regeneration is rare, it would seem that the production of carcinoma from a pre-existent squamous metaplasia also is rare. In the developmental process of squamous cell carcinoma, the main factor can be said to be the metaplastic metamorphosis of basal cells and columnar cells during or after cancerization.

It is extremely interesting that characteristic clear cells are seen in varying numbers within basal cell carcinoma and squamous cell carcinoma, and that they may occupy almost entirely the whole extent of the tumor. From the fact that these cells are seen frequently in the squamous carcinoma group, Ringertz[7] considered them to be probably a special degenerative form of squamous cells. I found 2 cases of solid or papillary clear cell carcinoma composed solely of these clear cells. In these tumors the peripheral areas of the alveolar structure consisted of polyhedral cells possessing a small, round, hyperchromatic nucleus with a single nucleolus and rich, clear, mucus-containing cytoplasm; and, as the central part was approached, a degenerative form of these was seen in which the cells were foamy or vacuolar, containing no mucus in the cytoplasm and having a pyknotic nucleus (Fig. 9). A point deserving special attention is the existence of small spindle cells which plainly display transition to the clear cells in the stromal boundary. Ringertz also reported the existence of a narrow zone of flattened cells with scanty cytoplasm and dark nuclei, which seems to be similar to my findings. However, I believe that the cytoplasmic change does not indicate a simple degenerative process, but a differentiation from basal cells into clear mucous cells, presumably as a feature in the multiplication of goblet cells. In relation to this phenomenon, it is of interest that basal cell carcinoma of the skin sometimes shows metaplastic goblet cell formation.[13] Recently Liu[14] reported 4 cases of clear cell carcinoma of the skin, in which it was believed that the origin of the clear cells can be traced to the basal cells, which are the matrix cells of the epidermis and skin appendages. The histologic structure of these cases indicated a rich glycogen content in the clear cells, as compared to the cases of paranasal tumors reported here in which such a phenomenon was not seen. Except for this, these groups resembled each other closely. This type of tumor grows slowly, and a low grade of destruction of the surrounding bony tissues was seen, but metastases were not demonstrable.

The mucous clear cells frequently are seen intermixed within the structure of squamous cell carcinoma (Fig. 10). This histologic feature

of paranasal carcinomas seems to correspond to "epithéliomas à double métaplasie de la parotide" of Masson and Berger,[15] to "mixed epidermoid and mucus-secreting carcinoma" of De and Tribedi,[16] and to "muco-epidermoid tumors of salivary glands" of Stewart, Foote, and Becker,[17] and can be interpreted as being due to the coexistence of two types of cells originating from the basal cells, namely, squamous and clear cells. According to Stewart *et al.* and Foote and Frazell,[18] the muco-epidermoid tumor of the salivary gland presumably has its origin in the excretory duct system proximal to the lobules, and in these tumors there is seen a third type of cell which is neither mucous nor epidermoid. In other words, there seem to exist "intermediate" or basal cells capable of differentiation into mucous, epidermoid, and squamous cells. It is interesting that these writers explained the pleomorphism of the muco-epidermoid group entirely as above, and surely with good reason. These clear cells may be seen in basalomatous formations and by degeneration their cytoplasm may become foamy or they may be empty, and thus the structure shows a cribriform or pseudo-glandular formation. It may further present a pseudo-papillary or a cystadenomatous pattern (Fig. 11).

When the principles and limits of variation in structure of carcinomas belonging to the first category are summarized, they can be represented diagrammatically as follows:




As the diagram shows, differentiations have been made mainly on the diverse potential plasticity of the basal cells, because this basis is necessary for an understanding of the polymorphic features of these tumors. However, this does not necessarily coincide with the view of Halpert and Pearson[19] that all lung cancers arise from undifferentiated "reserve cells" of the bronchial epithelium.

### 2. CARCINOMAS ORIGINATING FROM THE TRANSITIONAL PORTION OF THE EXCRETORY DUCT AND GLAND

Compared with the previous type, carcinomas originating from the transitional portion of the excretory duct and gland are seen much less frequently, and they accounted for only 11 per cent of all cases. In the

two "adenomas" examined, early cancerous changes were seen over all areas in one, and in part of the other. As Ringertz[7] has stated, it frequently is difficult to draw a clear line of differentiation between benign adenoma and adenocarcinoma.

Carcinomas of the following structural types were observed and were seen in various combinations in one and the same tumor:

a. Anaplastic adenocarcinoma
b. Solid or trabecular adenocarcinoma
c. Mucous cell adenocarcinoma
d. Adenoid cystic carcinoma
e. Clear cell adenocarcinoma and oxyphilic granular cell carcinoma
f. Myxo-fibrous adenocarcinoma (so-called mixed tumor type)
g. Adenocarcinoma with acanthomatous areas

All of these histologic patterns commonly are seen in tumors of the major salivary glands,[18,20] oral mucous membrane,[20,21] nasopharynx,[22,23] middle ear,[24] and other parts, so that no local specificity can be recognized. From times past, the term salivary gland tumor or tumor of salivary gland type usually has been applied to these tumors as representing a special type neoplasm of the nasal and paranasal cavities. Moreover, their origin has been traced to misplaced "aberrant salivary gland tissue," although the histogenesis of mixed tumor originating in the salivary gland itself sometimes is explained by Cohnheim's theory. However, I consider that it is natural for the salivary and paranasal tumors to present similar histologic appearances since they are derived from genetically and morphologically analogous matrix tissue. At least the majority of paranasal glandular tumors are not derived from aberrant salivary gland tissue but may be said to develop from the normally existing transitional portion of the excretory duct and glandular epithelium.

Mention will next be made of some of the interesting tumor types.

*Clear Cell Adenocarcinoma.* Clear cell adenocarcinoma is a variety in which a layer of tumor cells is arranged in glandular formation around irregular spaces or in trabeculae, and the stroma is composed of a small amount of connective tissue accompanied by slender capillaries (Fig. 12). The tumor cells have round or oval hyperchromatic nuclei of varying sizes situated in the basal part of the cells. The cytoplasm is abundant and clear, cuboidal or cylindric, and sometimes contains mucicarmine-positive substance. This mucous material often can be seen in the glandular spaces. In degenerated cells the cytoplasm appears much swollen, and foamy or vacuolated but without mucus,

and the nucleus becomes pyknotic. In other parts of the same tumor there may be seen solid trabecular, or glandular areas composed of oval, cylindric or polyhedral cells with small, round, hyperchromatic nuclei and abundant, eosinophilic, granular cytoplasm (Figs. 13 and 14). In other parts there may be seen areas in which these eosinophilic cells are found intermingled in a wedge-like manner within the masses of the clear cells. Each of these characteristic features belongs to clear cell adenocarcinoma possessing mucus-secretory ability and to oxyphilic granular cell carcinoma. The cells with eosinophilic granular cytoplasm show all forms of transition into the clear cells, whereby it becomes evident that the latter cells are derived from the former. This tumor does not contain other tissues such as epidermoid elements or lymphoid stroma. The clear cells are basically analogous to cells of the previously mentioned solid or papillary clear cell carcinoma, but there exists a difference in the structural pattern; namely, the clear cells of clear cell carcinoma imitate the goblet cells of the unicellular gland system, while those of the clear cell adenocarcinoma present the picture of ductal cavity formation, suggestive of origin from the excretory duct system. Of deep interest is their relation to the clear cell variants of salivary papillary cystadenoma,[20,25-28] the muco-epidermoid group,[17] and the oxyphilic granular cells.[29] In this respect it was noted that my case resembled closely the clear cell type of bilateral papillary cystadenoma of the parotid glands reported by Bauer and Bauer.[20] The cells which they described, however, were water clear cells showing negative reactions to mucus, fat, and glycogen staining, and differed from the cells reported here in that they did not contain mucus.

*Adenoid Cystic Carcinoma.* Billroth[30] was the first to employ the term cylindroma for a tumor presumably originating from the paranasal mucous glands, and since then tumors with the same histologic pattern have been found in other areas, such as the major and minor salivary glands, nasopharynx, pharynx, trachea, bronchi, skin, and mammary and lacrymal glands.[18] Here, problems arise regarding histogenesis and variation in histologic features. As to origin, both ducts and acini, especially the former, have been suspected, but no definite conclusion has been reached. I have had a case of cylindroma of definitely proved acinous origin, in which early malignant change arose multicentrically in the glandular portions (Figs. 15 to 17). Within certain gland spaces and cystic cavities were seen collections of mucus, indicating the mucus-secreting ability of the tumor cells. The same tumor showed great polymorphism in histologic pattern, and in some areas of the primary neoplasm as well as within the recurrent tumor the histologic pattern was that of oat-cell carcinoma, of simple

or trabecular adenocarcinoma, and of adenocarcinoma with pseudo-adamantine structure (Fig. 18).

*Columnar Cell Carcinoma with Adeno-acanthomatous Type.* Here there is seen in some areas the structure of columnar cell carcinoma, and in others an adeno-acanthomatous pattern with glandular formation and keratinizing squamous epithelium (Figs. 19 and 20). This type originates presumably from the transitional part of the excretory duct and shows traces on the one hand of differentiation into respiratory tract epithelium and on the other of the glandular formation of the excretory duct epithelium.

*Myxo-fibrous Adenocarcinoma.* All of the features associated with myxo-fibrous adenocarcinoma correspond to those of mixed salivary tumors, the state of the stroma and its relation to tumor cells being especially characteristic, as seen in Figures 21 and 22. However, in the 2 cases examined by me, the so-called cartilage-like tissue was not seen. The origin of tumors of this type seems also to be in the transitional portion of the excretory duct.

### 3. UNCLASSIFIED CARCINOMA

Unclassified carcinomas present chiefly the features of carcinoma simplex, and include tumors of obscure origin. Among them there are probably some in which minute examination of other parts of the same tumor may show characteristics traceable to one of the categories already mentioned. Hence the need arises for more minute and extensive histologic examination.

## SUMMARY

Upon 72 carcinomas, 4 papillomas, and 2 adenomas of the paranasal sinuses, studies of the histogenesis were made and classification attempted. As matrix tissues of paranasal carcinomas, two epithelial elements can be considered, and the tumors were divided into: (1) carcinoma originating from the surface epithelium and the major part of excretory ducts, (2) carcinoma originating from the transitional portion of excretory ducts and the glands, (3) unclassified carcinoma.

In the first category, the basal cells mainly account for the diversified cancerous pattern, and the variations in tumor types can be understood through the multiple potentialities of development of which basal cells are capable. The histogenesis of clear cell carcinoma and of squamous cell carcinoma proved to be of special interest, and hence detailed accounts of these features have been presented.

Carcinomas belonging to the second category, fewer in number, seemed to be derived mainly from the transitional portion of the ex-

cretory ducts. Almost all histologic structures seen in salivary gland tumors were seen in carcinomas of this region. I consider that the term salivary gland tumor should not be applied to tumors arising elsewhere than in the salivary glands, including the paranasal sinuses, as such use will lead to confusion. One case each of clear cell adenocarcinoma with oxyphilic granular cells, cylindroma of glandular origin, and adenocarcinoma with acanthomatous areas were reported here, as being of peculiar interest.

As has been pointed out by many previous writers, both papillomas and adenomas represent precancerous states, and such were studied for their evidences of early malignant proliferation. Those cases of paranasal carcinoma which presented only one histologic pattern were rare. The great majority were characterized by diversified patterns composed of two or more types of cancer. Hence, cases should not be diagnosed by microscopic examination of a limited part of the tumor.

I wish to thank Drs. H. Usizima and J. Tamura of the Department of Pathology, Nagoya University School of Medicine, for their generosity in providing me with materials. I am also indebted to Dr. S. Iwase for helpful suggestions in the histochemical studies. It is a pleasure to record here a debt of gratitude to Professor F. Oshima for his kindness in reading the original manuscript.

## REFERENCES

1. Cranmer, L. R. Malignant neoplasms of the paranasal sinuses. *A. M. A. Arch. Otolaryng.*, 1953, **58**, 704–709.
2. Mattick, W. L., and Streuter, M. A. Carcinoma of the maxillary antrum. *Surgery*, 1954, **35**, 236–242.
3. Marks, H. Inoperable carcinoma of antrum of Highmore. Report of a twenty-year survival after telecurietherapy and of a five-year survival after roentgen-grid therapy. *A. M. A. Arch. Otolaryng.*, 1954, **59**, 340–346.
4. Poulsen, I. P. V. Malignant tumours in nose and paranasal sinuses. *Acta Oto-laryng.*, 1953, **43**, 474–481.
5. New, G. B. Malignant diseases of the paranasal sinuses. *Am. J. Surg.*, 1938, **42**, 170–173.
6. Salinger, S. The paranasal sinuses. *A. M. A. Arch. Otolaryng.*, 1954, **60**, 203–240.
7. Ringertz, N. Pathology of malignant tumors arising in the nasal and paranasal cavities and maxilla. *Acta Oto-laryng.*, 1938, Suppl. 27, 405 pp.
8. Schaffer, J. Die Becherzellen. In: Handbuch der mikroskopischen Anatomie des Menschen, Möllendorff, W. v. (ed.). J. Springer, Berlin, 1927, **2**, Pt. 1, 107–120.
9. Maximow, A. A., and Bloom, W. A. Textbook of Histology. W. B. Saunders Co., Philadelphia, 1953, ed. 6, 616 pp.
10. Heiss, R. Nasenhöhle. In: Handbuch der mikroskopischen Anatomie des Menschen, Möllendorff, W. v. (ed.). J. Springer, Berlin, 1936, **5**, Pt. 3, 709–728.

11. Schaper, A., and Cohen, C. Beiträge zur Analyse des tierischen Wachstums: Über zellproliferatorische Wachstumszentren und deren Beziehungen zur Regeneration und Geschwulstbildung. *Arch. f. Entwcklngsmechn. d. Organ.*, 1905, **19**, 348–445. (Cited by Bauer.[20])

12. Geschickter, C. F. Tumors of the nasal and paranasal cavities. *Am. J. Cancer*, 1935, **24**, 637. (Cited by Ringertz.[7])

13. Willis, R. A. Pathology of Tumours. Butterworth, London, 1953, ed. 2, 997 pp.

14. Liu, Y. The histogenesis of clear cell papillary carcinoma of the skin. *Am. J. Path.*, 1949, **25**, 93–103.

15. Masson, P., and Berger, L. Epithéliomas à double métaplasie de la parotide. *Bull. Assoc. franç. p. l'étude du cancer*, 1924, **13**, 366–373.

16. De, M. N., and Tribedi, B. P. A mixed epidermoid and mucus-secreting carcinoma of the parotid gland. *J. Path. & Bact.*, 1939, **49**, 432–433.

17. Stewart, F. W.; Foote, F. W., Jr., and Becker, W. F. Muco-epidermoid tumors of salivary glands. *Ann. Surg.*, 1945, **122**, 820–844.

18. Foote, F. W., Jr., and Frazell, E. L. Tumors of the major salivary glands. *Cancer*, 1953, **6**, 1065–1133.

19. Halpert, B., and Pearson, B. The cellular structure of carcinoma of the lung; a study of 92 cases. *Am. J. Cancer*, 1940, **40**, 213. (Cited by Willis.[13])

20. Bauer, W. H., and Bauer, J. D. Classification of glandular tumors of salivary glands; study of 143 cases. *A. M. A. Arch. Path.*, 1953, **55**, 328–346.

21. Smith, A. G.; Broadbent, T. R., and Zavaleta, A. A. Tumors of oral mucous glands. *Cancer*, 1954, **7**, 224–233.

22. Cappell, D. F. The pathology of nasopharyngeal tumours. *J. Laryng. & Otol.*, 1938, **53**, 558–580.

23. Hara, H. J. Malignant tumors of the nasopharynx. Transpalatine surgical excision. *A. M. A. Arch. Otolaryng.*, 1954, **60**, 440–452.

24. Brunner, H. Basal-cell carcinoma of the external auditory canal and middle ear. *A. M. A. Arch. Otolaryng.*, 1953, **58**, 665–676.

25. Hückel, R. Eine ungewöhnliche Adenomform der Parotis. *Verhandl. d. deutsch. path. Gesellsch.*, 1930, **25**, 342–347.

26. Franssen, R. Eine parathyreoidea-ähnliche Geschwulst der Ohrspeicheldrüsen. *Zentralbl. f. allg. Path. u. path. Anat.*, 1932–33, **56**, 113–115.

27. Škorpil, F. Über das Cystadenoma papillare der grossen und kleinen Speicheldrüsen. *Frankfurt. Ztschr. f. Path.*, 1941, **55**, 39–59. (Cited by Bauer.[20])

28. Rawson, A. J.; Howard, J. M.; Royster, H. P., and Horn, R. C., Jr. Tumors of the salivary glands; a clinicopathological study of 160 cases. *Cancer*, 1950, **3**, 445–458.

29. Thompson, A. S., and Bryant, H. C., Jr. Histogenesis of papillary cystadenoma lymphomatosum (Warthin's tumor) of the parotid salivary gland. *Am. J. Path.*, 1950, **26**, 807–849.

30. Billroth, T. Beobachtungen über Geschwülste der Speicheldrüsen. *Virchows Arch. f. path. Anat.*, 1859, **17**, 357–375. (Cited by Foote.[18])

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[ *Illustrations follow* ]

## LEGENDS FOR FIGURES

FIG. 1. Case 75 (58-year-old female). An initial stage of papilloma with respiratory epithelium, found in association with inflammatory polyposis. × 100.

FIG. 2. A typical papilloma with respiratory epithelium from a recurrence of the tumor of case 75. × 100.

FIG. 3. Case 50 (47-year-old female). Papilloma with transitional epithelium and no definite histologic evidence of carcinoma. × 100.

FIG. 4. Papillary squamous cell carcinoma with distinct keratinization, as seen in a recurrence of the tumor of case 50 about 6 months later. × 100.

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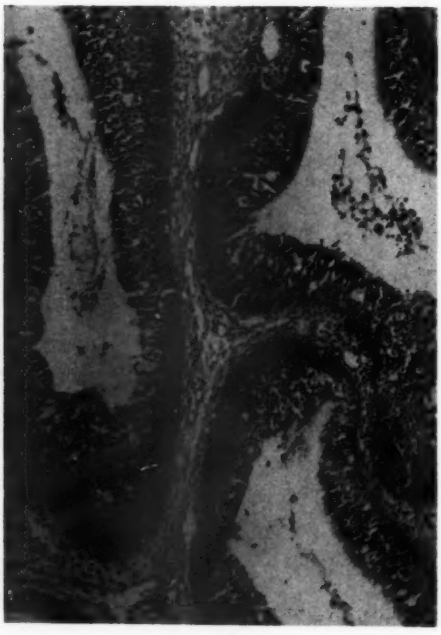

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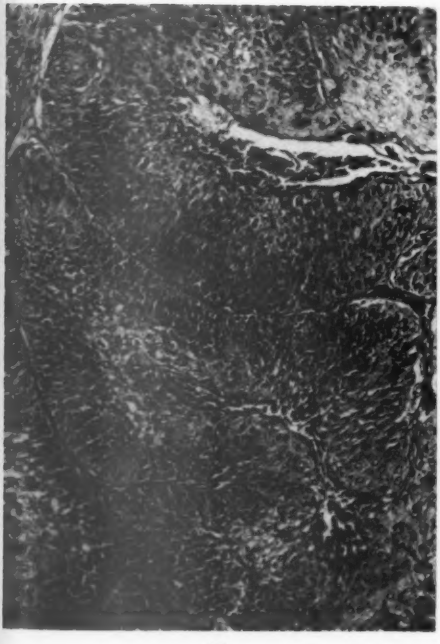

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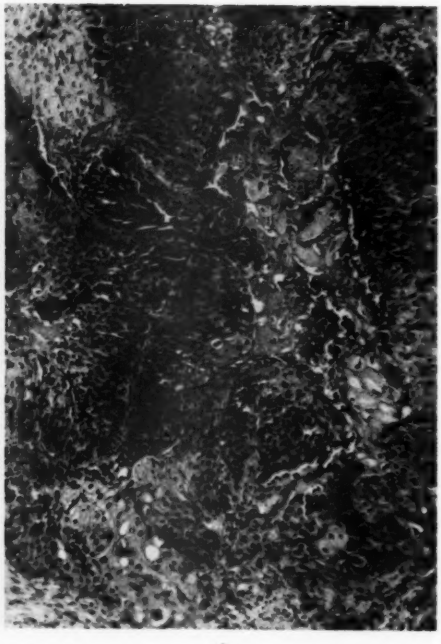

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FIG. 5. Case 54 (49-year-old male). Basal cell carcinoma composed of basophilic, small spindle cells, as seen in the primary tumor of the maxillary sinus. × 400.

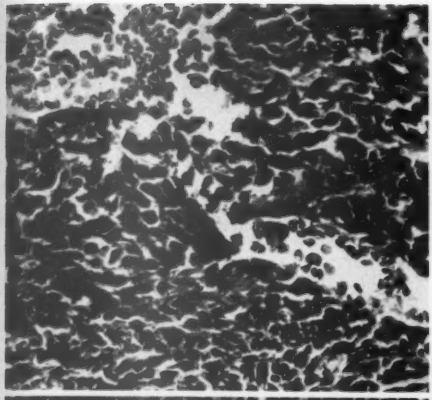
FIG. 6. Case 54. Small round cell carcinoma metastatic in a testicle, showing diffuse proliferation. These lymphocyte-like cells are regarded as of anaplastic type derived from the basal cells. × 400.

FIG. 7. Case 78 (53-year-old female). A basal cell carcinoma composed of large, elongated, spindle cells. × 400.

FIG. 8. A pattern of papillary carcinoma with columnar epithelium in the same tumor as that from which Figure 7 was taken, showing signs of low-grade malignancy histologically. × 400.

FIG. 9. Case 13 (45-year-old male). Clear cell carcinoma showing solid structures bordered by a thin zone of stroma. The clear cells in the peripheral part contain mucicarmine-positive material, but those in the central part show a foamy structure without mucus. × 200.

FIG. 10. Case 7 (47-year-old male). Muco-epidermoid carcinoma with cells laden with abundant mucus. × 200.



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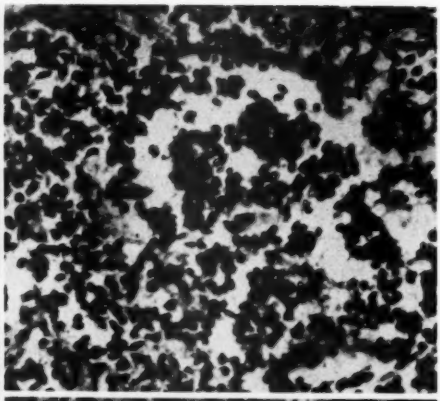

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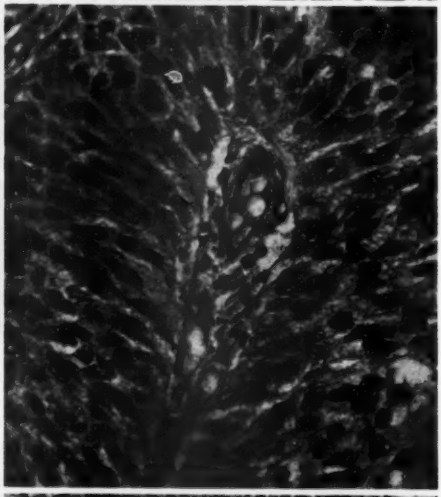

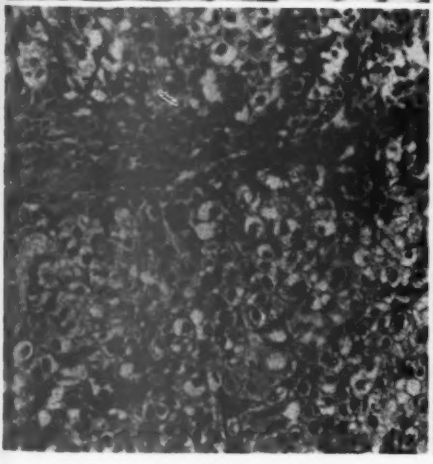

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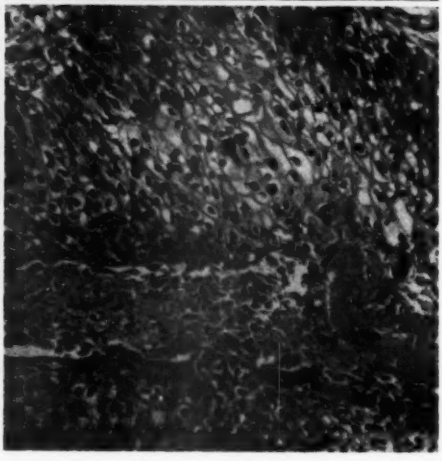


FIG. 11. Case 12 (45-year-old male). On the right, there is a cribriform or pseudo-glandular formation of basal cell carcinoma, and on the left pseudo-papillary or adenoid cystic pattern. The latter may result from the falling out of clear cells after they have become foamy. × 200.

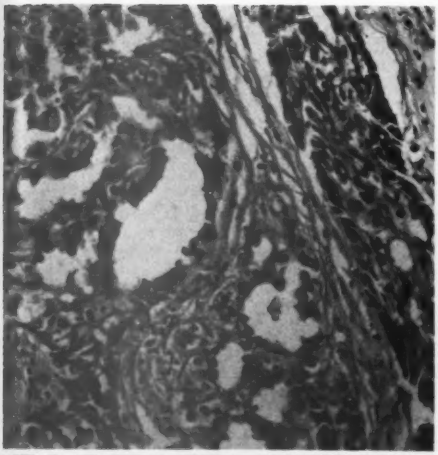
FIG. 12. Case 8 (48-year-old female). Clear cell adenocarcinoma, showing glandular structures lined by a layer of clear cells which occasionally contain mucus. × 200.

FIG. 13. An area of oxyphilic granular cell tumor in the same neoplasm as that shown in Figure 12. × 200.

FIG. 14. Solid formation composed of cells of the same type as in Figure 13. × 400.

FIG. 15. Case 31 (45-year-old female). An initial stage of cancerous change in the terminal portions of glands. × 400.

FIG. 16. The pattern of cylindroma seen in another section of case 31. × 100.



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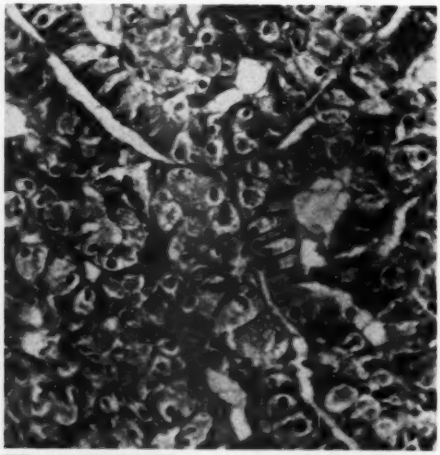

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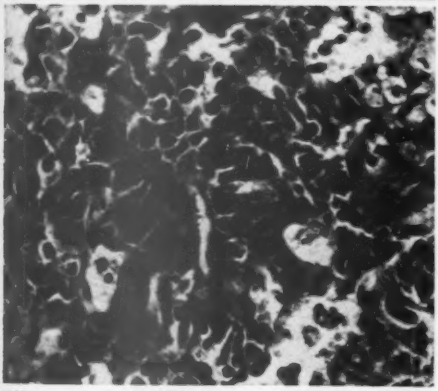

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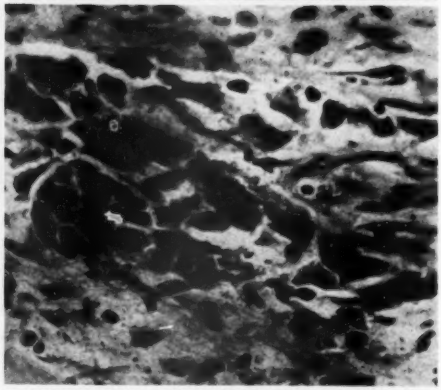

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FIG. 17. High-power view of cylindroma from case 31. × 400.

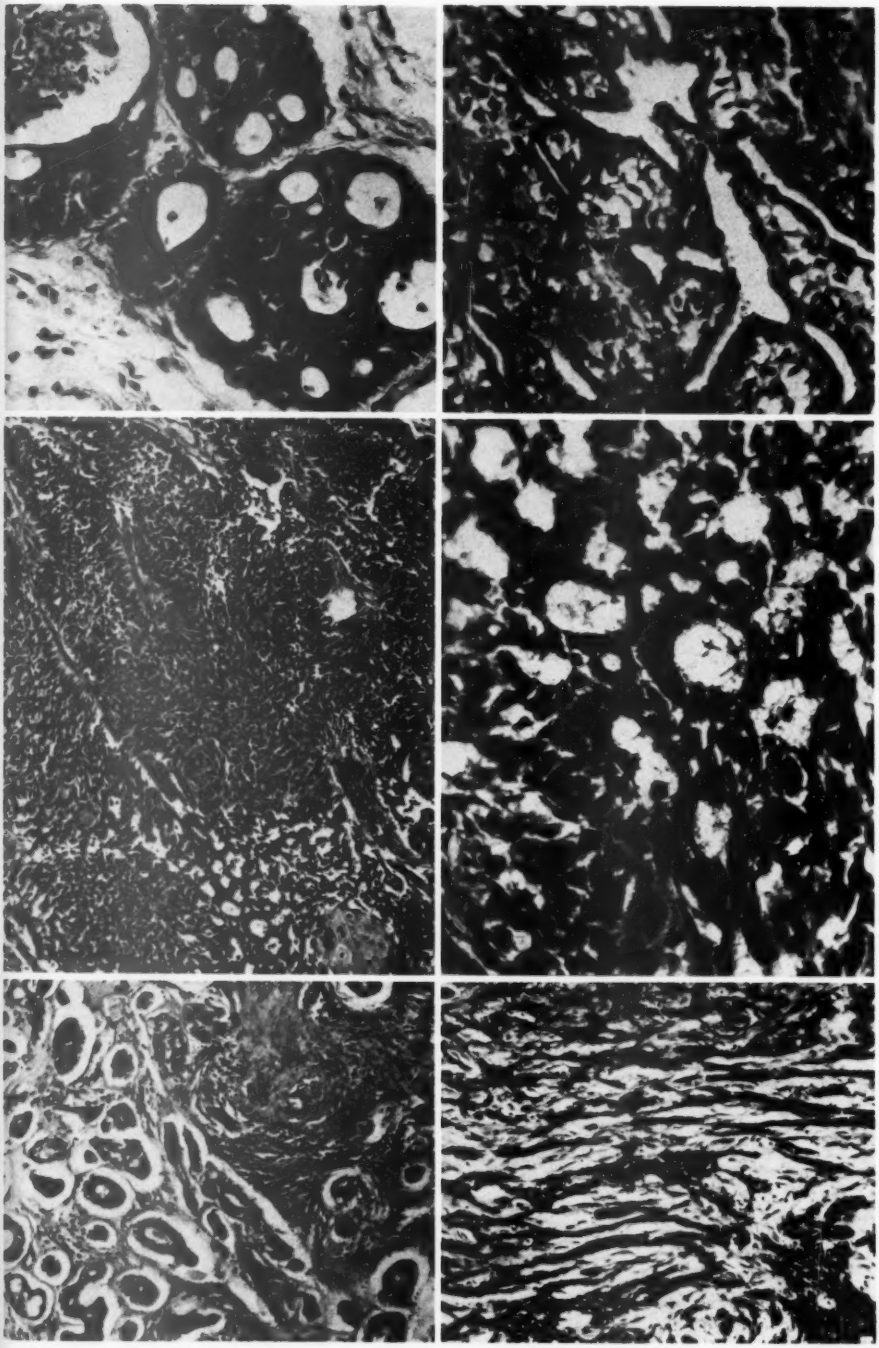
FIG. 18. A feature somewhat similar to the structural pattern of adamantinoma as seen in the same tumor as shown in Figure 17. × 400.

FIG. 19. Case 47 (41-year-old male). Columnar cell carcinoma with adeno-acanthomatous pattern. × 100.

FIG. 20. High-power view of glandular formation in Figure 19. × 400.

FIG. 21. Case 53 (64-year-old female). Glandular formations in a myxo-fibrous adenocarcinoma containing mucus in its cavities. × 100.

FIG. 22. A trabecular arrangement in the same specimen as that from which Figure 21 was taken. The stroma looks characteristically myxo-fibrous. × 100.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# SENILE AND SEBORRHEIC KERATOSES

## Localization of Succinic Dehydrogenase, Protein-Bound Sulfhydryl, and Disulfide Groups *

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The effects of aging in the skin are obvious, psychologically important, and accessible to study, but comparatively little is known of their pathogenesis, prevention, or retardation.[1] Knowledge of the histology of these changes is not extensive. Senile keratoses (keratomata senilis) are among the most important changes occurring in this aging process, since they are definitely precancerous.[2] They appear usually on the exposed surfaces of the body, particularly on the face and backs of the hands.[3] In spite of their importance, relatively few investigations of senile keratoses have been undertaken.[4] Among the lesions which must be differentiated from senile keratoses clinically and histologically are seborrheic keratoses (verruca senilis, verruca pigmentosum, pigmented papilloma, basal cell papilloma).[5] These occur particularly on the trunk and forehead and frequently are found in association with senile keratoses.[2] This differentiation is of considerable practical importance because of the far lesser malignant potentiality of seborrheic keratoses.

As a contribution to knowledge of the aging process in the skin, the localizations of succinic dehydrogenase activity, protein-bound sulfhydryl, and disulfide groups, all having relationship to growth, metabolism, or maturation of epidermal cells, have been studied in a series of senile and seborrheic keratoses.

Succinic dehydrogenase plays a vital rôle in the respiratory processes of most living cells and forms a link in the chain of reactions concerned with the oxidation of lipids, carbohydrates, and proteins.[6] Sites of succinic dehydrogenase activity have been found to be correlated with regions of cell growth in various contexts. In the uterine cervix, manifestation of this enzyme activity occurred in the basal layer of

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Portions of this work were done in the Departments of Pathology and Biochemistry, the University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

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normally maturing portio vaginalis epithelium, but was found more diffusely through metaplastic epithelium and in squamous cell carcinoma.[7]

Protein-bound sulfhydryl groups are essential for the activity of many enzymes.[8] They serve as protein links and have an important function in cell division and growth.[9] The presence of sulfhydryl groups in the epidermis is closely related to the process of keratinization where they undergo a transformation to disulfide substances in the stratum corneum and are concerned with cell division and proliferation.[10]

In the present investigation, histochemical localization of these substances has been studied in senile and seborrheic keratoses of human skin. Dehydrogenase activity was present in considerable degree in non-keratinized cells, less in keratinizing cells, and absent in regions of keratinization. Disulfide reaction occurred in areas of keratinization. Sulfhydryl reaction was found in all epidermal cells.

## Materials and Methods

Tissue blocks to include adjacent skin were obtained from 18 examples of senile keratosis and 7 examples of seborrheic keratosis. Dehydrogenase activity was localized by incubating fresh tissue blocks under reduced oxygen tension at 37° C. for 1 hour in solutions containing 0.23 per cent neotetrazolium,[7] 0.81 per cent sodium chloride, 0.03 molar sodium succinate, and 0.08 molar phosphate buffer, pH 7.4. Control blocks were incubated in a similar solution except that 0.03 molar sodium malonate was used instead of sodium succinate to inhibit the succinic dehydrogenase reaction.[11] Frozen sections (15 $\mu$) were used.

The technique of Barrnett and Seligman[12] was employed for sulfhydryl localization. Disulfide was detected by treating sections overnight with 0.1 molar sodium iodoacetate to block pre-existing sulfhydryl groups and, after washing, immersing the slides in a solution containing 10 per cent sodium acetate and 5 per cent sodium sulfite. After washing, the procedure for sulfhydryl localization was applied. Sections from all blocks were stained with hematoxylin and eosin for comparison.

## Results

Sites of succinic dehydrogenase activity were indicated by cytoplasmic deposition of black or brown crystals or granules of formazan.[13] Areas of weaker reaction occasionally revealed red microdroplets rather than crystals.[14] Control blocks incubated in malonate-containing solutions showed much less reaction. The specific reaction

in the sulfhydryl and disulfide preparations was indicated by red to blue staining of cytoplasm and nucleoli.[12]

Adjacent epidermis was studied for comparison in every case.[15] Formazan deposition manifesting dehydrogenase activity was heaviest in the basal and malpighian layers of the epidermis. It was less prominent in the granular layer and was absent in keratinized cells. Sulfhydryl reaction was found in the cytoplasm and nucleoli of the epidermal cells in the basal, malpighian, and granular layers. It was somewhat heavier in keratinized surface epithelium. The disulfide reaction was comparatively weak in the deeper epidermal layers. As intracellular keratinization occurred, moderate disulfide reaction appeared. A strong blue staining for disulfide was seen in keratinized surface epidermis.

The histopathologic features of the senile keratoses (Figs. 1 to 4) were as conventionally described.[2] The various lesions presented microscopic patterns ranging from comparatively innocuous hyperkeratoses through increasing degrees of cellular atypism to keratoses associated with developing squamous cell carcinoma manifesting minimal invasion. In the basal and malpighian layers there was considerable evidence of dehydrogenase activity in the cytoplasm (Fig. 2). In the granular layer there was a considerable diminution of formazan deposition. None was found in keratinized cells. Sulfhydryl reaction (Fig. 3) was found in nucleoli and cytoplasm in the basal and malpighian layers and in the granular layer. This reaction was stronger in keratinized cells. Disulfide reaction (Fig. 4) was present in basal, malpighian, and granular layers but was much weaker than the sulfhydryl reaction. Disulfide reaction was much stronger in keratinized cells than in non-keratinized cells. Keratohyaline granules showed no particular reaction to any of these techniques.

The seborrheic keratoses were predominantly of the keratotic type[5] with cystic inclusions of horny material surrounded by sheets of squamous cells (Figs. 5 to 8). Thick interwoven tracts of "basal cells" formed the bulk of the lesions. These "basal cells" showed considerable evidence of dehydrogenase activity (Fig. 6). The squamous cells lying adjacent to the keratin cysts had lesser formazan deposition. No evidence of dehydrogenase activity was found in the keratin pearls or in surface keratin. The predominant "basal cells" had cytoplasmic and nucleolar reaction to the sulfhydryl technique (Fig. 7). This reaction was somewhat stronger in keratinizing squamous cells and in nonviable keratin as seen in the cysts and on the surface. Disulfide reaction was comparatively weak in the "basal cells" and heavier in the cystic inclusions of horny material (Fig. 8).

## Discussion

Each of the substances studied is important in varied aspects of cell function and growth. Succinic dehydrogenase is an important link in the chain of enzymes which are responsible for biologic oxidation.[16] In the current study, sites of dehydrogenase activity correspond generally with regions of growth in squamous cells, being found in cells whose structural features suggested relationship to basal or malpighian layers. Less evidence of dehydrogenase activity was found in maturing squamous cells and none in epithelial pearls or regions of surface keratinization. Investigations by various workers support this correlation of dehydrogenase activity with sites of cell growth or active metabolism. Padykula[17] stated that the distribution pattern of succinic dehydrogenase suggests that this enzyme is present in greatest concentration within those portions of tissues and organs that show large metabolic capacity or are engaged in absorptive or secretory activity. Previously we found a distribution pattern in basal and squamous cell carcinoma of skin analogous to that currently described.[18] Distribution of dehydrogenase activity in squamous epithelium and in other tissues, as seen in various studies, certainly gives no evidence of correlation limited to any specific function. However, accumulated evidence appears to support the thesis that evidence of this enzyme group is a good index of general cell activity, whether it is physiologic[19] or neoplastic[7] growth, hormone secretion,[20] exocrine secretion (as in the endocervical glands),[21] or absorption (as in foreign body giant cells).[22]

Sulfhydryl groups are important in varied cell functions, in the coagulation of blood, in cell permeability, and in hormone synthesis and activity.[9] In experimental studies employing protozoa,[23] the sulfhydryl content of the cells increased progressively, reaching a maximum just before cell division. Normal epidermis contains in some of its layers a high concentration of protein-bound sulfhydryl groups which can be detected by histochemical means.[24] As epidermal cells mature and keratinization develops, disulfide groups become more prominent. The sulfur of the epidermis and its derivatives is present mostly as cysteine residues in the malpighian layer and cystine residues in the cornified layer.[25] At the limit between these two zones the sulfhydryl group of cysteine is oxidized to the disulfide bond of cystine, a process by which the keratin precursor present in the tonofibrils of the malpighian layer is transformed into the completed keratin present in the tonofibrils of the cornified layer.[26] Concomitant staining for sulfhydryl and for disulfide groups in varying degrees of

reaction, as found in the current study, is not unexpected, since sulfhydryl and disulfide provide an intracellular oxidation-reduction system in many tissues.[12] It has been suggested that free sulfhydryl groups disappear completely with the formation of keratins from their sulfhydryl-containing precursors.[26] However, Barrnett and Seligman[12] found a variable amount of disulfide and sulfhydryl in the keratinized zone. In the present investigation, sulfhydryl reaction was found in regions of keratinization.

Keratohyaline granules did not react with the techniques that we employed. Allen[27] stated that these granules are apparently desoxyribose nucleic acid. However, Rothman[28] believes that this relationship has not been established and that nothing is known about their nature.

Senile and seborrheic keratoses differ markedly in their biologic potential. Senile keratoses commonly are considered as precancerous,[1,4,5] since if they are left untreated, squamous cell carcinoma will develop in 20 to 25 per cent of them.[3] Examples of squamous cell carcinoma developing in senile keratosis are frequent in surgical pathologic material. The differences in microscopic pattern between an active senile keratosis and an early squamous cell carcinoma are those of moderate increase in cellular atypism with minimal invasion. An analogous relationship in microscopic pattern pertains between seborrheic keratosis and basal cell carcinoma, whose cells have marked similarity. From a practical viewpoint this cytomorphologic relationship is more apparent than real. Actual change of a seborrheic keratosis into a frank basal cell carcinoma with deep invasion and ulceration is rare.[5]

The histochemical reaction patterns of these two types of keratoses did not reflect their differences in growth potential. Cells of both lesions having some resemblance to those of the basal or malpighian layers of epidermis showed evidence of dehydrogenase activity and of sulfhydryl groups. Keratinizing cells from both lesions contained sulfhydryl and disulfide groups. This essential similarity in histochemical reaction pattern is not unexpected, since no striking and uniform histochemical differences have been demonstrated between neoplastic and non-neoplastic squamous epithelium.[29]

## Summary

The occurrence and distribution of dehydrogenase activity and of protein-bound sulfhydryl and disulfide groups were studied in senile keratoses and seborrheic keratoses, with the following results: (1) Dehydrogenase activity and sulfhydryl groups, both related to vital

phases of cell function and growth, were found in non-keratinized cells of both lesions, as well as in basal and malpighian layers of adjacent epidermis. (2) Evidence of dehydrogenase activity diminished in cells undergoing keratinization and was absent in regions of complete keratinization. (3) Disulfide groups, related to cell keratinization, and sulfhydryl groups were found in keratinizing cells and in regions of keratinization in senile keratoses, seborrheic keratoses, and in epidermis.

Despite the more ominous precancerous potential of senile keratoses, with these techniques their histochemical reaction pattern was essentially similar to that found in seborrheic keratoses.

The technical assistance of Miss Genevieve Marino and Mrs. Dorothy Schiller is gratefully acknowledged.

## REFERENCES

1. Allen, A. C. The Skin. A Clinicopathologic Treatise. C. V. Mosby Co., St. Louis, 1954, p. 45.
2. Ormsby, O. S., and Montgomery, H. Diseases of the Skin. Lea & Febiger, Philadelphia, 1954, ed. 8, p. 847.
3. Cooper, Z. K. In: Cowdry's Problems of Ageing, Lansing, A. I. (ed.). Williams & Wilkins Co., 1952, ed. 3, p. 784.
4. Cowdry, E. V., and Andrew, W. Some cytochemical and cytologic features of senile keratosis. *J. Gerontol.*, 1950, **5**, 97–111.
5. Lever, W. F. Histopathology of the Skin. J. B. Lippincott Co., Philadelphia, ed. 2, 1954, 518 pp.
6. Seligman, A. M., and Rutenburg, A. M. The histochemical demonstration of succinic dehydrogenase. *Science*, 1951, **113**, 317–320.
7. Foraker, A. G.; Denham, S. W., and Aguilar Celi, P. Localization of sites of dehydrogenase activity in the cervix uteri: normal, metaplastic, and neoplastic epithelium and cervicitis. *Cancer*, 1954, **7**, 311–317.
8. Barrnett, R. J., and Seligman, A. M. Histochemical demonstration of protein-bound sulfhydryl groups. *Science*, 1952, **116**, 323–327.
9. Barron, E. S. G. Thiol groups of biological importance. *Advances Enzymol.*, 1951, **11**, 201–266.
10. Montagna, W. The cytology of mammalian epidermis and sebaceous glands. *Int. Rev. Cytol.*, 1952, **1**, 265–304.
11. Quastel, J. H., and Wheatley, A. H. M. Biological oxidations in the succinic acid series. *Biochem. J.*, 1931, **25**, 117–128.
12. Barrnett, R. J., and Seligman, A. M. Histochemical demonstration of sulfhydryl and disulfide groups of protein. *J. Nat. Cancer Inst.*, 1953–54, **14**, 769–803.
13. Shelton, E., and Schneider, W. C. On the usefulness of tetrazolium salts as histochemical indicators of dehydrogenase activity. *Anat. Rec.*, 1952, **11**, 61–81.
14. Foraker, A. G., and Denham, S. W. Histochemical localization of succinic dehydrogenase and endogenous reductase activity in squamous cell carcinoma of the cervix. *Texas Rep. Biol. & Med.*, 1953, **11**, 643–652.

15. Foraker, A. G., and Wingo, W. J. Histochemical studies of skin: localization of dehydrogenase activity, protein-bound sulfhydryl and disulfide groups. *A. M. A. Arch. Dermat.*, 1955, **72**, 1–6.

16. Wachstein, M., and Meisel, E. Influence of experimental renal damage on histochemically demonstrable succinic dehydrogenase activity of the rat. *Am. J. Path.*, 1954, **30**, 147–165.

17. Padykula, H. A. The localization of succinic dehydrogenase in tissue sections of the rat. *Am. J. Anat.*, 1952, **91**, 107–145.

18. Wingo, W. J., and Foraker, A. G. Correlation of protein-bound sulfhydryl and disulfide groups and of succinic dehydrogenase activity in basal cell and squamous cell carcinomas of skin. *J. Histochem. & Cytochem.*, 1954, **2**, 472.

19. Foraker, A. G.; Denham, S. W., and Mitchell, D. D. Dehydrogenase activity. III. In the rabbit placenta. *Obst. & Gynec.*, 1954, **4**, 527–535.

20. Foraker, A. G.; Aguilar Celi, P., and Denham, S. W. Dehydrogenase activity. I. In the ovary. *Obst. & Gynec.*, 1953, **2**, 407–413.

21. Foraker, A. G., and Denham, S. W. Succinic dehydrogenase as an indicator of cellular metabolism in the cervices of pregnant and nonpregnant women. *Surg., Gynec. & Obst.*, 1953, **96**, 259–264.

22. Foraker, A. G.; Denham, S. W., and Aguilar Celi, P. Dehydrogenase activity. II. In the fallopian tube. *Obst. & Gynec.*, 1953, **2**, 500–507.

23. Barron, E. S. G. The importance of sulfhydryl groups in biology and medicine. *Texas Rep. Biol. & Med.*, 1953, **11**, 653–670.

24. Rudall, K. M. The proteins of the mammalian epidermis. *Advances Protein Chem.*, 1952, **7**, 253–290.

25. Rothman, S. Physiology and Biochemistry of the Skin. University of Chicago Press, Chicago, 1954, p. 368.

26. Giroud, A., and Leblond, C. P. The keratinization of epidermis and its derivatives, especially hair, as shown by x-ray diffraction and histochemical studies. *Ann. New York Acad. Sc.*, 1951, **53**, 613–626.

27. Allen, A. C. *l. c.*, p. 15.

28. Rothman, S. *l. c.*, p. 375.

29. Foraker, A. G. Comparison of histochemical properties of normal and neoplastic squamous epithelium. (Abstract.) *Am. J. Path.*, 1955, **31**, 581.

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[ *Illustrations follow* ]

## LEGENDS FOR FIGURES

FIGS. 1 to 4. Senile keratosis.

FIG. 1. Hematoxylin and eosin section. Keratohyaline granules are prominent in the stratum granulosum. × 171.

FIG. 2. Neotetrazolium section. Tissue, from the same case as that from which Figure 1 was taken, processed in neotetrazolium with succinate to show sites of dehydrogenase activity. Formazan granules are prominent in the cytoplasm of squamous cells in the deeper layers, but are absent in regions of keratinization. × 171.

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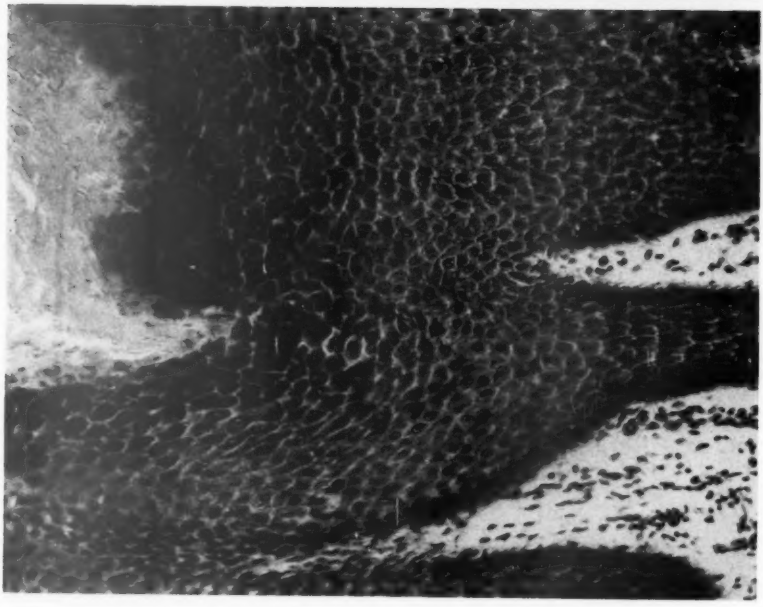

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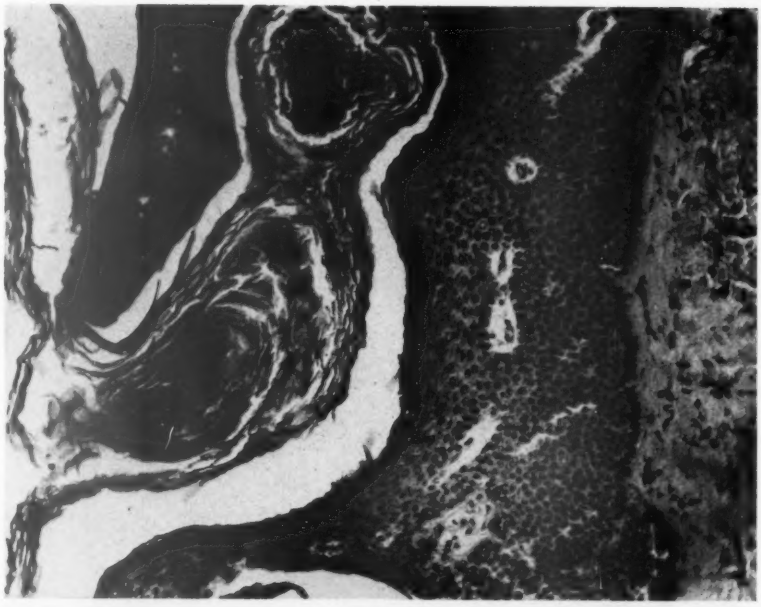
Fig. 3. Sulfhydryl preparation. Moderate staining for sulfhydryl groups is found in nucleoli and cytoplasm in the basal, malpighian, and granular layers. Heavier staining is present in regions of keratinization. × 171.

Fig. 4. Disulfide preparation. Very weak nucleolar and cytoplasmic reaction is found in nucleoli and cytoplasm in the basal, malpighian, and granular layers. Comparatively heavier reaction is present in keratinized cells. × 171.

Figs. 5 to 8. Seborrheic keratosis.

Fig. 5. Hematoxylin and eosin section. × 171.



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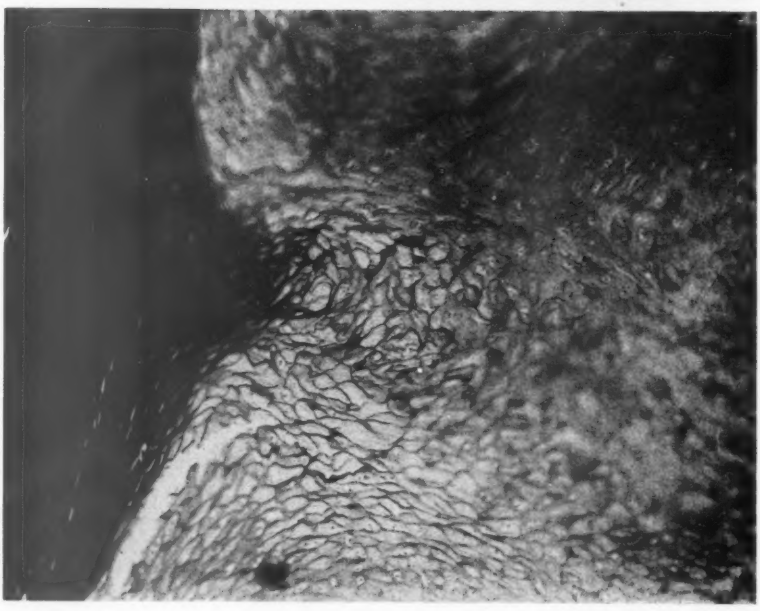

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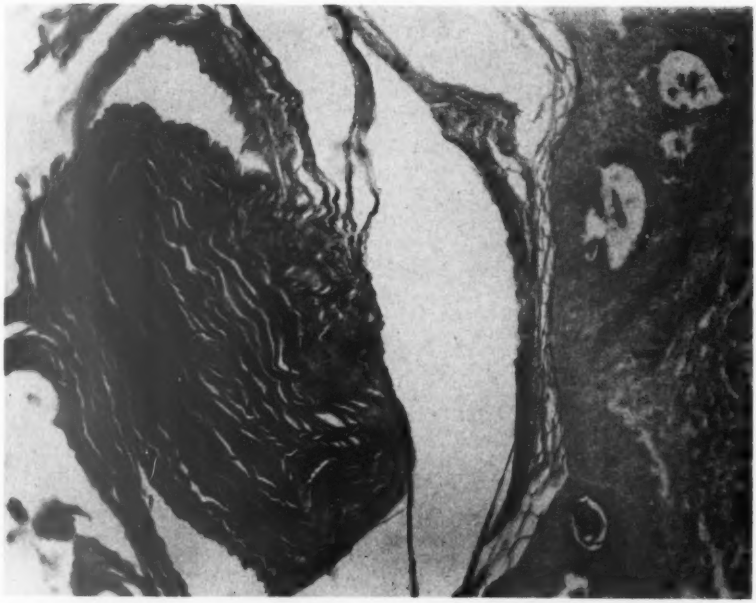
Fig. 6. Neotetrazolium section. Formazan crystals and granules manifesting dehydrogenase activity are found in the cytoplasm of non-keratinized epidermal cells. Evidence of dehydrogenase activity is not seen in areas of keratinization. × 171.

Fig. 7. Sulfhydryl preparation. Moderate sulfhydryl reaction is found in nucleoli and cytoplasm of non-keratinized cells, but slightly heavier in regions of keratinization. × 171.

Fig. 8. Disulfide preparation. Extremely weak reaction is present in cytoplasm and nucleoli of non-keratinized epidermal cells. Comparatively heavier reaction is present in regions of keratinization. × 171.



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# THE MICROSCOPIC APPEARANCE OF ECTHYMA CONTAGIOSUM (ORF) IN SHEEP, RABBITS, AND MAN *

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Ecthyma contagiosum, otherwise known as orf and contagious pustular dermatitis, is a virus disease of sheep which is occasionally transmitted to man. The microscopic appearance of the lesions of ecthyma contagiosum presents several unusual features which merit detailed description. The lesions will be described as they appear in sheep, rabbits, and man.

## ECTHYMA CONTAGIOSUM IN SHEEP

The microscopic appearance of the lesions of ecthyma contagiosum in sheep has been reported by Aynaud,[1] Lloyd *et al.*,[2] Pask *et al.*,[3] Asakawa *et al.*,[4] and Glover.[5] The descriptions of these authors are essentially in agreement with our own except they are not as detailed and do not stress the granulomatous and papillomatous features of the disease as much as would seem indicated.

The description of the disease that follows is based upon the inoculation of the virus of ecthyma contagiosum into the scarified skin of 6 sheep. Tissue was removed for biopsy on 80 occasions, ranging from the 4th to the 42nd day after inoculation of the virus.

On the fourth day after inoculation there was essentially no change in the epidermis and the dermis showed a small amount of vascular dilatation. By the fifth and sixth days the epidermis was mildly acanthotic and the uppermost four or five layers of cells in the prickle cell layer were undergoing ballooning degeneration. The dermal infiltrate was prominent and was composed largely of round cells and reticulo-endothelial cells. There were many newly formed blood vessels and their walls showed well developed, proliferative endothelial changes. The vascular changes remain a prominent feature of the disease throughout its course and they will not be mentioned again.

On the seventh and eighth days the ballooning degeneration of the uppermost portion of the prickle cell layer resulted in almost complete loss of nuclei and cytoplasm of the cells, and the remaining cell walls produced a "basket-weave" appearance. A few tiny, superficial vesicles were produced by the process of reticular degeneration. The

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underlying prickle cell layer presented mild pseudo-epitheliomatous hyperplasia and there was considerable dissolution of the basal layer. The dermal infiltrate was increased and there was edema of the upper portion of the dermis. At times there was enough fluid in the dermis to produce small subepidermal vesicles. By the eleventh day the surface of the lesion was composed of a superficially placed, multilocular pustule, whose roof was a thin horny layer, and whose base was the proliferating prickle cell layer. At the base of the pustule there remained a few scattered balloon cells. The underlying epidermis was moderately pseudo-epitheliomatous and the basal layer presented marked dissolution. The dermal infiltrate was very dense and composed largely of round cells and reticulo-endothelial cells or young fibroblasts. A few polymorphonuclear cells could be seen in the upper portion of the dermis.

Between the 11th and 17th days the cells of the pustule disintegrated and the surface of the lesion became covered with a heavy layer of hyperkeratotic and parakeratotic material. The epidermis showed marked pseudo-epitheliomatous hyperplasia and dissolution of the basal layer. The dense dermal infiltrate persisted. Between the 17th and 22nd days the lesion was covered with a dense crust which became progressively less parakeratotic and more hyperkeratotic. The rete pegs grew downward and the dermal papillae grew upward to produce a finger-like papillomatous appearance. The dermal infiltrate was composed of the same cell types but it was decreased slightly in amount. Less edema was evident and there was very little dissolution of the basal layer of the epidermis.

Between the 22nd and 40th days the lesion gradually involuted. The papillomatous character gradually disappeared and the dermal infiltrate gradually resolved. At the time the crust separated, the epidermis showed mild acanthosis and the dermis contained a minimal cellular infiltrate which was largely perivascular and periappendageal. Slight increase in connective tissue was present but this was not sufficient to be evident grossly as scar.

In summary, the early phases of the disease are characterized by ballooning degeneration of the uppermost portion of the prickle cell layer, which results in multiloculated, superficially placed vesicles and pustules which are replaced late in the disease by a heavy crust. The middle phase of the disease is characterized by pseudo-epitheliomatous hyperplasia and granuloma formation which gives way near the end of the disease to a papillomatous phase. The corresponding phases of the disease present grossly as red macules which develop successively

into red papules, gray pustules, and brown crusts which overlie warty, papillomatous bases.

Re-inoculation of the virus of ecthyma contagiosum into sheep (partially immune animals) produces essentially the same lesions grossly and microscopically. The re-inoculation disease lasts for a much shorter period. The pustular, crusted, and granulomatous phases appear much earlier and in most animals the papillomatous phase is not seen. When it does occur it is inconspicuous and of short duration.

### Ecthyma Contagiosum in Rabbits

Most authors[1,4,5] have failed to observe evidence of disease in rabbits after the inoculation of the virus of ecthyma contagiosum, but a few[6,7] have reported the production of a mild disorder. We have observed the appearance of tiny (1 to 2 mm.), erythematous maculopapules in rabbit skin after intradermal inoculation.[8] The lesions appeared after an incubation period of 7 to 9 days and the duration of the disease was 3 to 5 days. We performed three serial passages in rabbit skin and produced typical ecthyma contagiosum in a sheep inoculated with third passage rabbit material. The dilutions in these serial passages were great enough to make it seem quite certain that the virus was able to proliferate in the skin of the rabbit.

There is only one microscopic description of ecthyma contagiosum in rabbits to be found in the literature, to the best of our knowledge, and there is some doubt whether the disease described in that instance is ecthyma contagiosum.[9]

Our description of the microscopic appearance of ecthyma contagiosum in rabbits is based upon the intradermal inoculation of the virus in 8 animals. During three serial passages of the virus the microscopic appearance of the lesions did not change. Under low magnification the lesions usually appeared flat, but an occasional section showed very mild papillomatosis. Higher magnification showed no specific change in the epidermis (occasional insignificant crusting and slight spotty decrease in thickness). The dermis contained a round-cell infiltrate which varied in amount from moderately dense to very dense. Some edema and vascular dilatation were evident and there was a moderate amount of new blood vessel formation and proliferation of the endothelial lining of these vessels.

The lesions in rabbits are characterized microscopically by mild chronic inflammatory changes in the dermis and lack of ballooning degeneration and the prominent granulomatous and proliferative character of the disease in sheep. One might expect such findings from the

gross appearance of the lesions which are tiny, evanescent, erythematous maculopapules.

## Ecthyma Contagiosum in Man

Descriptions of the microscopic appearance of ecthyma contagiosum in man can be found in the writings of Robert and Orbaneja,[10] Kingery and Dahl,[11] Percival *et al.*,[12] and Pask *et al.*[3] It is probable, too, that Stark *et al.*[13] have described the disease in some detail under the term milkers' nodules. The limited number of microscopic examinations of human lesions of ecthyma contagiosum make a complete description of the disease impossible for any one observer, but a good idea of the histologic picture can be gained from a compilation of the findings of the several authors, including an analysis of the material available to us.[14]

The epidermis became thickened, largely through proliferation of the cells of the prickle layer. The cells of this layer underwent ballooning degeneration and, by an extension of the process (reticular and colliquative degeneration), vesicles were formed in the epidermis. A considerable degree of parakeratosis often developed and at times pseudo-epitheliomatous hyperplasia was found. Inclusion bodies might or might not be seen in smears prepared from the vesicles.[2,3,6]

The dermis presented edema and dilatation of the blood vessels and lymphatics. New blood vessel formation was prominent and the endothelial cells of many of the vessels showed active proliferation. Varying amounts of dermal infiltrate were seen, but usually there was a dense collection of lymphocytes and reticulo-endothelial cells among which could be found a few polymorphonuclear leukocytes and plasma cells.

The microscopic picture, therefore, resembles lesions produced by the pock viruses (ballooning degeneration leading to vesiculation) with added findings that usually are associated with a chronic granuloma. The microscopic picture is consistent with the appearance of the gross lesions which are often multiloculated vesicles and at other times resemble pyogenic granulomas.

## Summary

The inoculation of the virus of ecthyma contagiosum into the skin of sheep, rabbits, and man produces lesions which are different in many respects. In sheep, ballooning degeneration and the formation of superficial, multiloculated vesicles and pustules are characteristic of the early phases of the disease and granulomatous and papillomatous

features predominate in the later phases. The disease in rabbits does not show ballooning degeneration and it lacks the pronounced granulomatous or papillomatous character of the lesions in sheep. Lesions in man usually are characterized by vesiculation, which is a result of ballooning degeneration of the cells of the prickle layer, but a granulomatous character occasionally dominates the microscopic appearance. These differences in the microscopic features in the three species constitute an excellent example of the important part the reaction of the host plays in the disease picture produced by an infectious agent.

The virus of ecthyma contagiosum usually is classified with the pock-producing dermatropic viruses (herpes simplex, herpes zoster, vaccinia, variola, varicella). There is some justification for this classification because ballooning degeneration is a prominent feature of the lesions in sheep and man. The consistent granulomatous and papillomatous features of the lesions in sheep, the lack of a pock-like picture in rabbits, and the tendency of the lesions to become granulomatous in man, however, set ecthyma contagiosum apart from the usual pock disorders.

## REFERENCES


1. Aynaud, M. La stomatite pustuleuse contagieuse des ovins (chancre du mouton). *Ann. Inst. Pasteur*, 1923, **37**, 498–527.
2. Lloyd, G. M.; MacDonald, A., and Glover, R. E. Human infection with the virus of contagious pustular dermatitis. *Lancet*, 1951, **1**, 720–721.
3. Pask, V. M.; Mackerras, I. M.; Sutherland, A. K., and Simmons, G. C. Transmission of contagious ecthyma from sheep to man. *M. J. Australia*, 1951, **2**, 628–632.
4. Asakawa, Y.; Imaizumi, K.; Tajima, Y., and Endo, M. Studies on a contagious ecthyma-like disease observed among the sheep. *Jap. J. M. Sc. & Biol.*, 1952, **5**, 475–486.
5. Glover, R. E. Contagious pustular dermatitis of the sheep. *J. Comp. Path. & Therap.*, 1928, **41**, 318–340.
6. Blakemore, F.; Abdussalam, M., and Goldsmith, W. N. A case of orf (contagious pustular dermatitis): identification of the virus. *Brit. J. Dermat.*, 1948, **60**, 404–409.
7. Blanc, G., and Martin, L. A. Sensibilité du lapin et de l'homme au virus de la stomatite des ovins. *Compt. rend. Acad. d. sc.*, 1933, **197**, 586–587.
8. Wheeler, C. E.; Potter, M., and Cawley, E. P. Experimental ecthyma contagiosum. *J. Invest. Dermat.* In press.
9. Selbie, F. R. Shope papilloma and sheep dermatitis in the rabbit: mutual interference, superinfection and effects of chemical pre-treatment of the skin. *Brit. J. Exper. Path.*, 1946, **27**, 143–154.
10. Robert, P., and Orbaneja, G. Trois cas de granulomes angiopapillomateaux éruptifs infectieux. *Ann. de dermat. et syph.*, 1937, **8**, 45–55.
11. Kingery, L. B., and Dahl, J. Ecthyma contagiosum in man. Data concerning its incidence in several Western states; report of a case. *Arch. Dermat. & Syph.*, 1945, **51**, 359–364.

12. Percival, G. H.; Drennan, A. M., and Dodds, T. C. Atlas of Histopathology of the Skin. E. & S. Livingstone, Ltd., Edinburgh, 1947, pp. 187 and 197.

13. Stark, A. M.; Tresenhausen, M. M.; Gozanskaja, N. M.; Skrozky, D.; Schtastny, D. S., and Zuk, W. A. Über die Pockenätiologie der sog. Melkerknoten. *Arch. f. Dermat. u. Syph.*, 1934, 170, 38–60.

14. Wheeler, C. E.; Cawley, E. P., and Johnson, H. A. Ecthyma contagiosum (orf). *A. M. A. Arch. Dermat.*, 1955, 71, 481–485.

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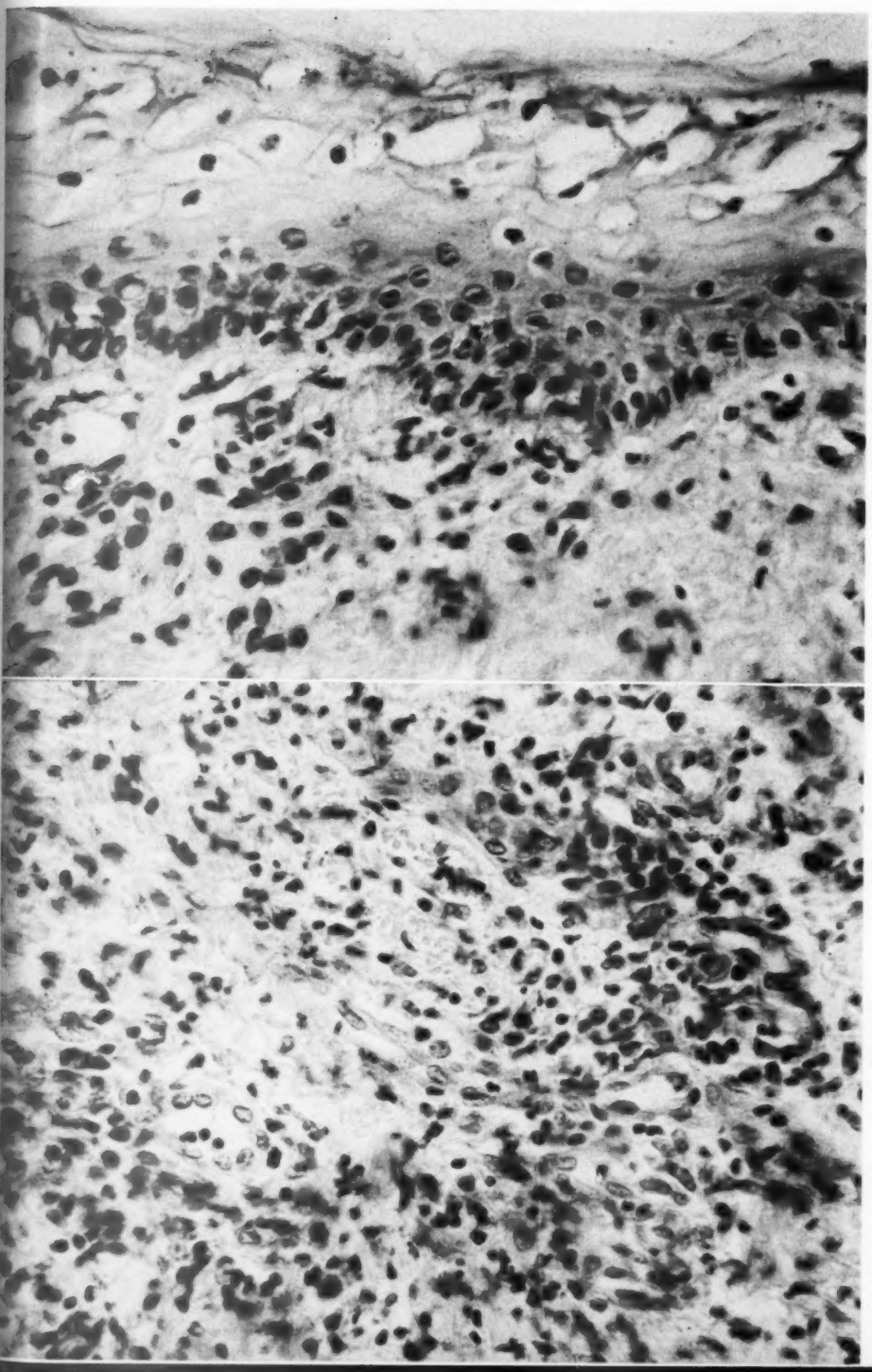
## LEGENDS FOR FIGURES

FIG. 1. Sheep V. Five days after inoculation of the virus of ecthyma contagiosum. Ballooning degeneration is present in the uppermost cells of the prickle layer and vascular changes and a sparse round-cell infiltrate can be seen in the dermis. × 445.

FIG. 2. Sheep II. Ten days after inoculation of the virus. The granulomatous changes in the dermis are well developed. × 445.



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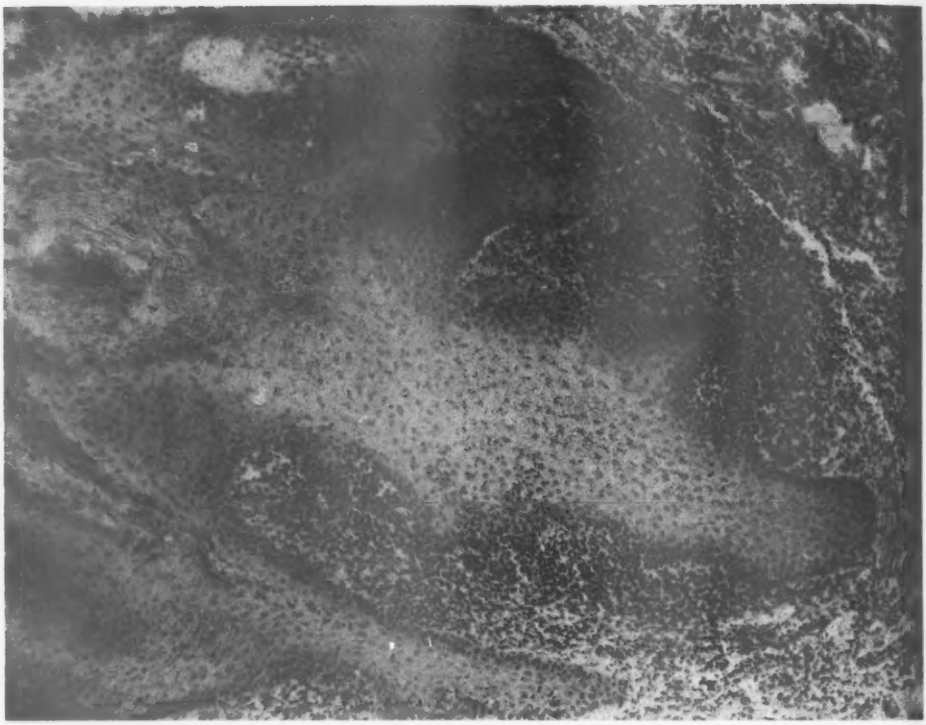
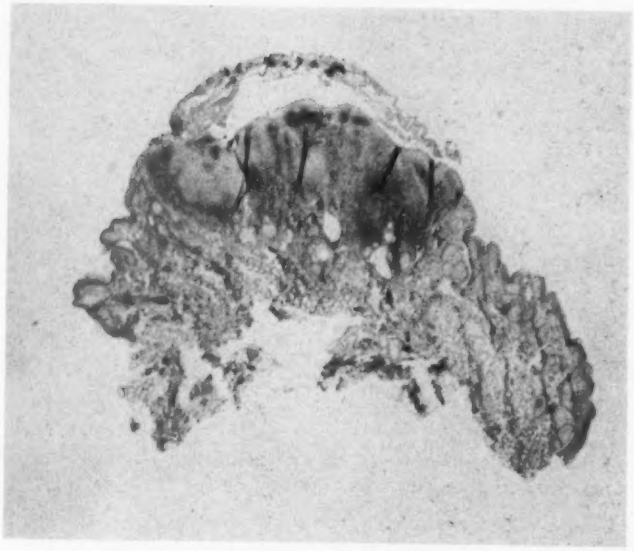


FIG. 3. Sheep V. Twelve days after inoculation of the virus. Pseudo-epitheliomatous hyperplasia is a prominent feature. × 110.

FIG. 4. Sheep II. Ten days after inoculation of the virus. This low-power photomicrograph (× 11) shows the superficial position of the pustule. Most of the pus cells have been lost in preparing the specimen.

FIG. 5. Sheep I. Seventeen days after inoculation of the virus. The low-power photomicrograph (× 9) shows the pronounced papillomatosis characteristic of the lesion at this age.



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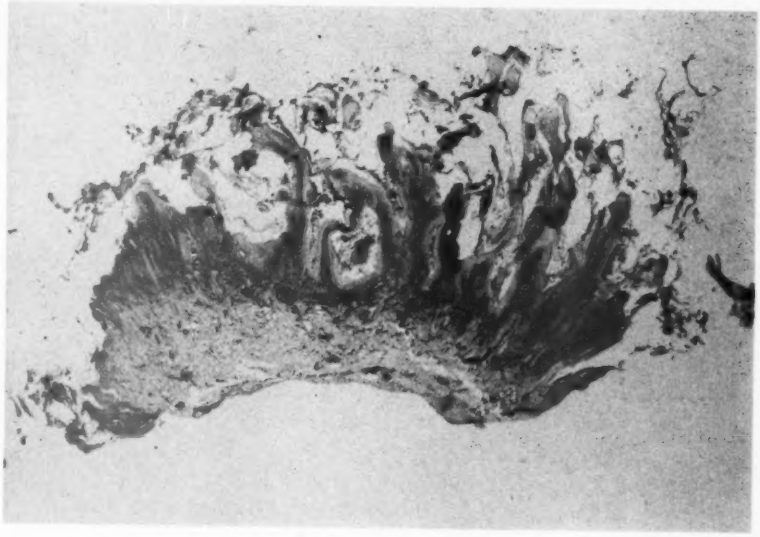

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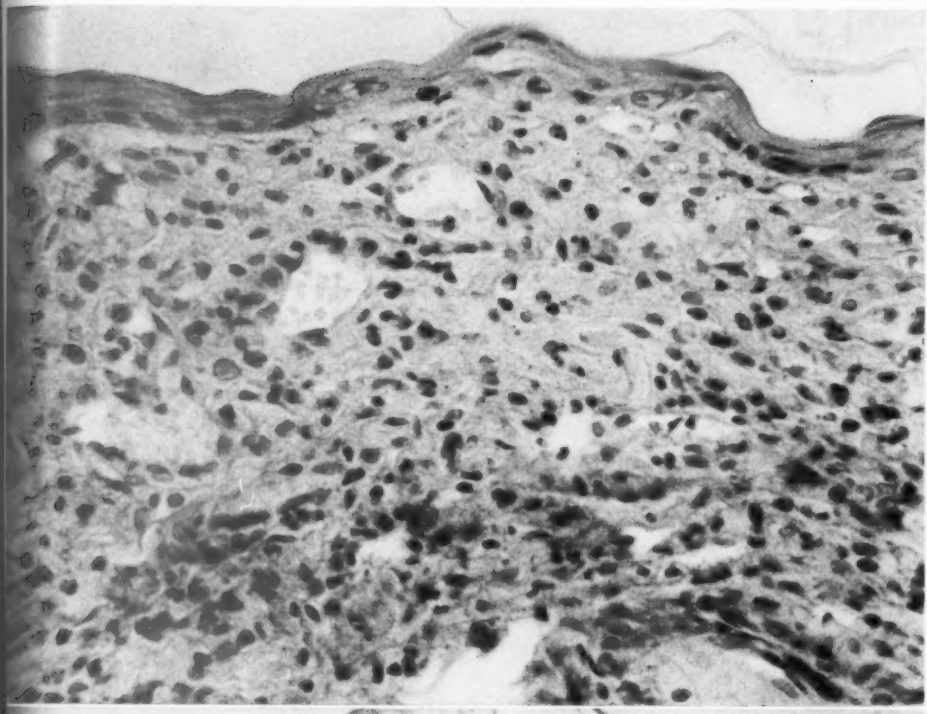
FIG. 6. Rabbit V. Third-passage lesion 9 days after inoculation of the virus. The lesion is essentially a granuloma. × 445.

FIG. 7. Human lesion approximately 3 weeks after inoculation of the virus. Ballooning and reticular degeneration have resulted in a multilocular vesicle. × 270.



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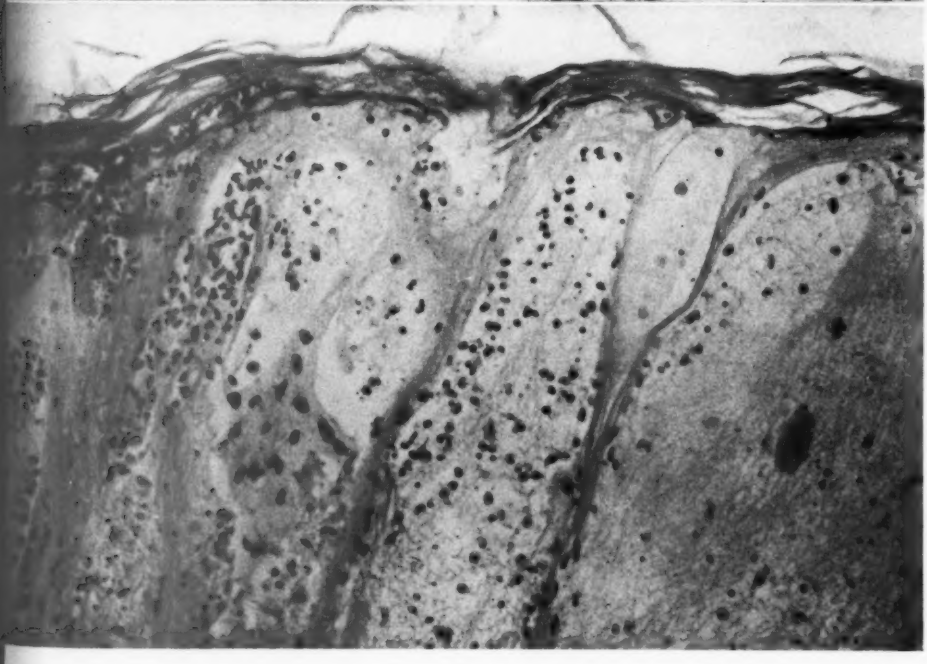

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# HISTOLOGIC EVIDENCE OF DEVELOPMENTAL LESIONS IN TEETH ORIGINATING FROM PALEOLITHIC, PREHISTORIC, AND ANCIENT MAN *

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A century ago Czermák[1] discovered in microscopic sections of human teeth the so-called interglobular dentin spaces (Figs. 1 and 2). The pathologic significance of this condition remained uncertain until the late twenties when Mellanby[2] reported her conclusive experiments indicating that comparable defects could be produced in dogs' teeth by introducing deficiencies in the calcifying property of the diet fed to the animals during the period of tooth development. Since then it has become well established that the human dentition, during formation and calcification, is very sensitive to nutritional as well as to many other metabolic disturbances. However, it is noteworthy that such defects are exceedingly rare in certain subhuman primates such as the wild rhesus monkey. In the course of the last 10 years I have prepared numerous sections of the teeth of these animals and have consistently found a remarkably uniform and perfect microstructure of the dentin and enamel of their teeth (Figs. 3 and 4).

It has been a matter of speculation whether the teeth of man might have been of a superior developmental structure in former times and in various parts of the world, whenever and wherever dental caries is known to have been absent, infrequent, or less prevalent than today. This problem appeared to be both interesting and feasible to explore. Not only do teeth retain permanent histologic evidence throughout the life of an individual regarding the original developmental quality, but teeth are at the same time sufficiently well preserved for long periods of time after death to be prepared for microscopic sections by means of special histologic procedures.[3]

The present investigation represents an exploratory histologic survey of the developmental quality of human teeth originating from earlier periods of time and various parts of the world; namely, Paleolithic Palestine, prehistoric Greece, predynastic Egypt, ancient Iceland, and middle age Norway from the old world, and Guatemalan and Pecos Indian teeth from the new world (Table I). Further details regarding the material and methods have been recorded in a previous report,[4] in which various post-mortem changes in the histologic appear-

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ance of the teeth have been differentiated and ruled out prior to this study of *intra vitam* features.

## Paleolithic Palestine

The teeth from the Old Stone Age which have been examined in this study originate from the fossil human remains found in two caves (Skhūl and Tabūn) on the western slope of Mount Carmel, Palestine,

Table I

*Source, Type, and Number of Teeth Examined*

| Origin | Incisor | Canine | Premolar | First molar | Second molar | Third molar | Total |
|---|---|---|---|---|---|---|---|
| Prehistoric Palestinian | 1 | 1 | | 4 | 1 | | 7 |
| Predynastic Egyptian | | 1 | 1 | | 1 | 1 | 4 |
| Ancient to Modern Greek | 15 | 12 | 44 | 17 | 5 | 17 | 110 |
| Old Icelandic | | | | 2 | | | 2 |
| Middle Age Norwegian | 10 | 3 | 6 | 2 | | 2 | 23 |
| Pecos Indian | 37 | 1 | 8 | 18 | 5 | 7 | 76 |
| Guatemalan Indian | 2 | 1 | 5 | 2 | 1 | | 11 |
| Total | 65 | 19 | 64 | 45 | 13 | 27 | 233 |

excavated by a joint British and American expedition between 1929 and 1937. The skulls and skeletons have been thoroughly studied and described by McCown and Keith[5] from the standpoint of gross features.

It is believed that the Mount Carmel population lived through the last interglacial period into the first phase of the last (Würm) glaciation. According to a recent monograph on human evolution by Clark,[6] these skeletal remains appear to represent a transitional stage leading from pre-Mousterian *Homo sapiens* to the later established *Homo neanderthalensis*.

The Skhūl material comprised a total of 10 individual skeletons, I to X, as well as 16 isolated fragments. From the Tabūn cave the remnants of 2 individuals were recovered in addition to half a dozen miscellaneous fragments. Altogether the Mount Carmel material included approximately 200 teeth. Of these there were about 30 isolated teeth from the Skhūl site and about 25 from the Tabūn site, the latter including a few teeth which were found in the superficial layers, probably not of Paleolithic origin. Through the courtesy of the late Professor Earnest Hooton of Harvard's Peabody Museum, 7 of the

teeth have been made available for the present histologic examination: 2 from the Skhūl cave and 5 from the Tabūn site, including 2 from the superficial layers.

*Skhūl Teeth*

The 2 teeth obtained from Skhūl were isolated molars from skeleton no. II in the series of 10 in Harvard's Peabody Museum. This skeleton is thought to be from an adult female, age 30 to 40 years, and consisted of fragments of the skull with 4 isolated teeth, the mandible with 6 isolated teeth, and humeri, radii, and ulnae. Found in layer B of the Skhūl site, containing a lower Levalloiso-Mousterian industry, these skeletal remains were of an Upper Pleistocene age, but nevertheless older than the Upper Levalloiso-Mousterian industry of layer B in the Tabūn cave. The 2 teeth placed at our disposal for microscopic examination were the mandibular right second molar (no. 25 in the Peabody record), illustrated in Figures 5 to 8 of the present paper, and the maxillary right first molar (no. 26 in the Peabody record), illustrated in Figures 9 and 10 of the present paper.

The mandibular molar is of particular interest because of its pathologic features. McCown and Keith[5] recorded the following measurements in millimeters: mesio-distal diameter of crown, 11.3; labio-lingual diameter, 10.0; crown height, 4.7; root length, 9.5; and total tooth length, 18.5. They also have presented a drawing of the buccal and lingual aspect of these teeth in their Figures 141 C and D respectively.*

When obtained from the Peabody Museum, this tooth weighed 1.8001 gm. Considerable wear was evident on occlusal, mesial, and distal surfaces (Fig. 5). The outside enamel and dentin, furthermore, showed evidence of post-mortem erosion. Mesially, a shallow cavity was observed involving both enamel and dentin at the neck of the tooth (Fig. 5).

At low power several dark patches could be found in the dentin, and surface erosions also were present in both the root surface and enamel surface. These defects may be attributed to previously described post-mortem changes.[4] At one point on the distal surface, near the neck of the tooth, was found a lesion which in part, at least, may have been of *intra vitam* origin. In view of the typical location, the involvement of the enamel, the depth of penetration, and the absence

* For the sake of future cross reference, it is important to note two typographic errors in the reference to this tooth in McCown and Keith's[5] book: on page 203, the illustrations of this tooth should have been referred to as Figure 141 C, D and not as 140 C, D; on page 212, Table 68 II, in the far right column the tooth should have been designated as a lower right second molar, not as an upper left second molar.

of typical post-mortem canals in the dentin at the cavity bottom, there may have been a small primary carious involvement (Figs. 5 and 6).

Structurally, the development of this tooth was extremely poor, particularly the dentin (Figs. 7 and 8). As many as 30 to 40 interglobular dentin spaces could be counted within certain areas, some of them very large, leaving widely segregated globules (Fig. 8). Considering the type of tooth (second permanent molar) and the peripheral location of the defects, it is believed that the mechanism of calcification failed at about 4 to 6 years of age in the life of this middle-aged woman. Within the enamel were found several accentuated incremental lines (Fig. 7) which probably were laid down simultaneously with, or just prior to, the defective dentin.

The maxillary first molar gave the following measurements in millimeters: mesio-distal diameter of crown, 9.9; labio-lingual, 11.7; height of crown, 6.0; length of root, 6.7; and total tooth length, 17.7. Drawings of the buccal and distal aspects of this tooth are reproduced in McCown and Keith's[5] Figures 140 C and D, respectively.

When obtained from the Peabody Museum (no. 26 in that collection), this tooth weighed 1.7761 gm. Marked attrition was present on occlusal as well as mesial and distal surfaces. Considerable post-mortem erosion had occurred on the root surface, which appeared yellowish brown, and on the interproximal and cervical portion of the enamel. Roentgenograms taken in bucco-lingual and mesio-distal directions indicated that the size of the pulp chamber had been decreased by secondary dentin, probably as a result of the extensive attrition. The roots appeared fairly short. In addition, the tip of the roots transmitted x-rays in a manner suggesting post-mortem changes.[4] This was confirmed by the histologic examination which revealed post-mortem canals invading the dentin from outside and inside. Erosion also occurred both on the root and the enamel surface (Fig. 9).

Histologic evidence of developmental disturbances was found. Several pronounced incremental lines were seen in the occlusal one third of the enamel (Fig. 9). Being a first permanent molar, it is evident that these lines must have developed during the first to second year of life. In the dentin just below this portion of enamel were observed several rows of interglobular dentin spaces (Fig. 10), as many as 10 being observed within a field of 1 sq. mm.

It is evident, therefore, that the woman to whom these teeth belonged (Skhūl II) must have suffered from some dietary or metabolic disturbance affecting the mechanism of calcification in at least two periods between birth and the age of 7 years.

### *Tabūn Teeth*

Six specimens from the Tabūn cave, all permanent teeth, were obtained for microscopic study, a molar from Miscellaneous Series I (Peabody's no. 9); an incisor and a first molar from Miscellaneous Series III (Peabody's nos. 19 and 17, respectively); and a canine and a molar from Miscellaneous Series V, found in more superficial deposits.

The tooth from Miscellaneous Series I, a first molar from an adolescent individual, came from layer B of the Tabūn site, which contained an Upper Levalloiso-Mousterian industry belonging to the Upper Pleistocene period. For this particular tooth McCown and Keith[5] (their Table 64 and page 209) have recorded the following measurements in millimeters: mesio-distal diameter of crown, 11.2, and of the neck of the tooth, 8.0; labio-lingual diameter of crown, 11.3, and of the neck, 10.7; height of crown, 6.9; length of root, 13.4; total tooth length, 19.7 mm.; dimensions which fall somewhat below corresponding Krapina and Le Moustier teeth. In addition they have presented drawings of the mesial aspect of this tooth (their Fig. 125 D) showing roots "well separated and no taurodontism," as well as a photograph showing the occlusal aspect of this and neighboring teeth (Fig. 1 of their Plate XIV).

When obtained from the Peabody Museum, this specimen weighed 2.1528 gm., which is within the normal range for dry maxillary first molars. No gross defects or hypoplasia were evident, but when magnified photographically, minute roughnesses could be observed in the surface texture of the enamel. In addition it was seen that the external root surface, when further magnified, exhibited a number of circular lines or growth rings. Roentgenographs of the tooth, taken in bucco-lingual and mesio-distal directions, revealed, in my opinion, a relatively tall pulp chamber.

In microscopic ground sections, cut in the long direction of the tooth, the enamel exhibited several accentuated incremental lines (Fig. 11), suggesting that the late possessor of this tooth suffered from some metabolic upset during the period of infancy and childhood. A failure in the mechanism of calcification definitely occurred at approximately 2 years of age, as indicated by the presence of interglobular dentin spaces, especially in the peripheral crown portion (Figs. 12 and 13). An extremely scalloped appearance of the dentin-enamel junction was noted (Fig. 13), a condition which we have found comparably extreme in specimens of Pecos Indian teeth which will be described.

The teeth from Miscellaneous Series III, a maxillary left central

incisor (Peabody no. 19) and a mandibular left first molar (Peabody no. 17) came from Tabūn's layer B, originating from the Upper Pleistocene period as evidenced by an Upper Levalloiso-Mousterian industry.

The central incisor showed both incisal and interproximal attrition, and must have belonged to a young adolescent individual. According to McCown and Keith[5] (their Table 64 and page 209), this tooth gave the following dimensions in millimeters: mesio-distal diameter of crown, 9.2, and of neck, 6.5; labio-lingual diameter of crown, 8.2, and of neck, 7.3; crown height, 10.0; root length, 17.0; and total tooth length, 25.8. The appearance of the lingual and mesial aspect of this tooth (their Figs. 121 C and 121 $C^1$, respectively) suggest a Neanderthalian tendency towards assuming a bicuspid form. When obtained from the Peabody Museum, this tooth weighed 1.4094 gm. On the incisal edge dark dentin was exposed by attrition. Some post-mortem etching was present on both the enamel and root surfaces, and the root appeared yellowish brown.

Ground sections of this tooth appeared yellow, but otherwise no post-mortem defects were present. Further examination of the sections made it evident that the great labio-lingual width of this tooth—its bicuspid tendency in other words—was caused entirely by the shape and size of the dentin and not by any excessive thickness of enamel, which appeared of average width, both labially and lingually. The growth lines in the enamel were relatively regular, but a few of the lines were more pronounced than the rest, corresponding to a calcification period of about 1½ to 2 years. Part of the enamel greatly obstructed the light, especially that formed after about 2 years of age. Other areas, especially the central portion, were very clear and transparent, as if the interprismatic substance had fused with the prisms, a condition similar to that observed in mottling of enamel.

The dentin showed a few moderately accentuated incremental lines (Fig. 14). By their location these lines must have arisen at about the age of 2 years. The condition is similar to the so-called O en's contour lines, already noted in Figure 1, in which the calcifying globules are not completely fused. On the lingual aspect the dentin-enamel junction was deeply scalloped. A small layer of secondary dentin was found below the mesial attrition zone. There was some granularity of the dentin but no pronounced or large interglobular dentin spaces in this tooth. The several depressions observed externally along the labial enamel surface would therefore appear to have been formative defects while calcification had proceeded somewhat more satisfactorily.

The mandibular left first molar from the same series belonged to a child between 6 and 12 years of age. Half of the roots had broken off,

and there was moderate attrition of the occlusal and mesial surface and absence of distal wear on the tooth. McCown and Keith[5] (their Table 64 and page 209) have recorded the following dimensions in millimeters: mesio-distal diameter of crown, 12.3, and of neck, 10.5; labio-lingual diameter of crown, 11.5, and of neck, 10.5; crown height, 8.1, and crown width, 93.5 per cent of length. The morphologic pattern of the crown of this tooth, which had four cusps and was approximately square, has been surveyed in detail by McCown and Keith[5] (their Fig. 129 B and Plate XIV, 12, pages 193 and 194) and is doubtlessly Neanderthaloid. Its surface texture was somewhat uneven with folded occlusal fissures and a moderately depressed transverse groove in the cervical portions of the enamel surface. On the buccal surface at the neck of the tooth the enamel showed a slight V-shaped extension between the roots.

Histologically, this tooth exhibited no significant post-mortem changes. In the cervical part of the dentin a row of 5 small interglobular spaces could be counted within a 1 sq. mm. field, corresponding to the portion calcifying at about 2 years of age. In another segment of the tooth, near the occlusal surface, which calcifies at about ½ year of age, as many as 20 interglobular dentin spaces could be found within a 1 sq. mm. field. The enamel had a somewhat pronounced incremental line in the occlusal portion, about two thirds of the distance from the dentin, and a number of distinct tufts extended from the dentin-enamel junction (Fig. 15). The cervical segment showed a much more transparent glossy enamel. Here the growth lines were inconspicuous. A diffuse prism structure was noted in the external one third of the enamel, i.e., the latest formed portion.

The teeth from Miscellaneous Series V, a mandibular left canine and a maxillary right first molar, originated from the superficial deposits on the terrace in front of the Tabūn cave and cannot be considered paleoanthropic, because these deposits contained material of Bronze Age to recent type, as well as derived Paleolithic implements and fossils from layer B. McCown and Keith[5] gave no measurements of these teeth.

The canine weighed 1.6542 gm. when obtained from the Peabody Museum; the mesio-distal diameter measured 7.9 mm., and the labio-lingual diameter, 9.3 mm. Attrition had reduced the crown height by several millimeters and part of the root was fractured. The weight, therefore, is of no significance, but if reconstructed, the total length may be estimated to have been about 27 mm. Judging by the limited wear, the canine is believed to have come from a young adult. Grossly, several growths rings could be seen on the root surface and a slight

roughness in the enamel surface near the neck of the tooth. Histologically, the enamel exhibited a number of very pronounced incremental lines (Fig. 16). Correspondingly, the dentin of this tooth, especially the periphery of the crown, showed a wide row of interglobular dentin spaces (Fig. 17), as many as 20 to 30 being seen within a 1 sq. mm. field. By their location (Fig. 18) it is evident that a failure in calcification occurred at the age of about 3 years in the life of the possessor of this tooth.

The molar weighed 2.7253 gm., mesio-distal diameter was 12.0 mm., and labio-lingual diameter, 10.9 mm., whereas total length and weight could not be determined because of crown wear and root fracture. The enamel showed a shallow transverse groove at the neck of the tooth. Between the roots this tooth showed a distinct V-shaped prolongation of the enamel. Because of the extensive attrition on occlusal as well as mesial and distal surfaces, this tooth is believed to be from an older individual. Histologically, two pronounced incremental lines were present in the cervical portion of enamel. The portion of dentin which normally calcifies between birth and 1 year of age was very defective (Fig. 19) with 15 to 30 interglobular spaces seen within a 1 sq. mm. area.

### Predynastic Egypt

From the Peabody Museum's collection of Predynastic Egyptian skeletal remains found at Keneh, Upper Egypt, in 1914, Professor Hooton made available for this study 4 specimens—a maxillary left second permanent molar (Peabody's no. 59002), belonging to the remains of a 16-year-old girl; a maxillary right third permanent molar, a mandibular right first permanent premolar, and a mandibular right canine—all adult teeth (Peabody's no. 58865).

The second molar was intact grossly, except for the tips of the roots which were partly broken. The surface was relatively rough with a brownish stain, part of which could be washed off, revealing quite deep pits and fissures and several cracks in the enamel surface. Upon sectioning, the enamel appeared pigmented when viewed in transmitted light, and a number of pronounced incremental lines could be seen. The dentin was moderately granular in appearance, with a few relatively small interglobular dentin spaces.

The third molar had marked attrition of all cusps, but the fissures were still distinguishable. The surface of the enamel was remarkably white and shiny, but the roots had all been broken off. Upon histologic examination, the enamel of this tooth appeared essentially normal, both in color and with regard to the distribution of the incremental growth lines. The dentin also was of a relatively good quality with the

exception of a peripheral granularity and a few isolated and small interglobular dentin spaces (Fig. 20).

The premolar tooth had been worn to the extent that no cusps were left. The root was intact, and the surface of the crown was relatively smooth with the exception of a few fracture lines, possibly of post-mortem origin. Histologically, the enamel was slightly pigmented, but no pronounced incremental lines were seen. The dentin had a number of scattered interglobular dentin spaces, but all of small dimensions.

The canine was also well worn, with a somewhat rough enamel surface. In this tooth there were not only several quite pronounced incremental lines in the enamel, but the dentin had very distinct interglobular dentin spaces—as many as 20 within a 1 sq. mm. field, suggesting that a failure in calcification had existed in the early life of the adult individual to whom this tooth once belonged.

### Prehistoric to Recent Greece

Through the courtesy of Professor John Koumaris, Director, Anthropological Museum, University of Athens, 86 teeth were provided for this study, with specimens from prehistoric to recent times. The same collection of skulls from which these tooth samples were obtained had previously been examined with regard to caries by Krikos,[7] who demonstrated a tremendous increase in the incidence of this disease during the same span of time. In addition to this material, 24 teeth from present Greece were obtained from the island of Thassos, through the kind cooperation of Dr. Nicos E. Efthyvoulides of Thessaloniki.

#### *Surface Texture*

Grading the surface texture of the enamel met with certain difficulties because the oldest teeth had extensive attrition and occasional post-mortem erosions; and the most recent teeth could be graded only in part because portions of the crowns were lost as a result of tooth decay. Of 35 prehistoric teeth, which could be most reliably graded, 3 showed full-fledged gross hypoplasia; more than half exhibited minor grooves and pits in the enamel surface, requiring that these be classified as showing the so-called M-hypoplasia; only 7 of these ancient teeth could be graded as perfect with regard to developmental quality as reflected by external enamel smoothness. Of 23 teeth originating from Archaic to Posterior age, 2 teeth showed gross hypoplasia, 19 exhibited M-hypoplasia, while only one tooth was perfectly normal with regard to surface texture. Of 23 teeth from recent time, 17 revealed M-hypoplasia. Of the 21 teeth which could be graded from the present time, 18 showed M-hypoplasia. In other words, the more

perfectly smooth enamel surface was less frequently encountered in the teeth from recent and present periods than in the earlier periods. Yet, at no time within the past 5,000 years under study did these Greek teeth exhibit a perfect developmental surface texture of the enamel.

### *Histologic Structure*

A more reliable index of the developmental structure of the teeth can be obtained through microscopic examination. The presence of uncalcified interglobular spaces in abnormally developed dentin offers an opportunity for semi-quantitative appraisal of the degree of developmental disturbance. The results of such a comparison are shown in Table II. From the histologic standpoint very few of the Greek teeth could be classified as being perfectly developed.

The prehistoric teeth represent a group which were developed from

TABLE II

*Results of Histopathologic Examination of Greek Teeth from Various Periods*

| Period | Number of teeth examined | Number with deficient structure | Average degree of defects*(mean) | Standard error of the mean | Critical ratio |
|---|---|---|---|---|---|
| Prehistoric (3000 B.C.–750 B.C.) | 40 | 30 | 5+ | 0.6 | |
| Archaic–Classic (750 B.C.–338 B.C.) | 12 | 10 | 7+ | 1.1 | 1.3 |
| Hellenistic–Roman (388 B.C.–325 A.D.) | 7 | 6 | 7+ | | |
| Posterior (330 A.D.–1800 A.D.) | 4 | 4 | 9+ | | |
| Recent (1800 A.D.–Recent) | 23 | 22 | 9++ | 1.7 | 2.2 |
| Contemporary (1948) | 24 | 22 | 20+++ | 1.0 | 12.9 |

* Average number of interglobular dentin spaces within a comparable field (1 sq. mm.). The plus signs represent an estimate of the degree of the developmental defects, e.g., the relative size of the interglobular dentin spaces.

about 3,000 B.C. to 750 B.C. Within this comparatively long period we do not have exact information on the relative age of each tooth concerned, but 3 of the oldest, known to have originated before 2,000 B.C., had a normal structure. Of the 40 teeth from the prehistoric Greek period, 30 showed some evidence of developmental defects, with an average of 5 interglobular dentin spaces per unit of tooth substance examined within a standard field. More often the dentin had a granular to areolar appearance, as illustrated in Figure 21, a first permanent molar from Asine, Peloponnesus, of prehistoric origin.

The 47 teeth from recent and contemporary periods were of deficient

microscopic structure in all but 3 cases. The degree of developmental defects, using the same unit as before, ranged from 9 in recent time to 20 in the small contemporary sample. Statistical comparison of these semi-quantitative data for the 47 teeth from recent and contemporary periods and for the 40 teeth from prehistoric time gave a standard difference between the means of 1.9 and a critical ratio of 4.5. A further breakdown of the data indicates a very significant difference between the structure of the prehistoric versus the contemporary specimens (critical ratio, 12.9). Descriptive observation of ground sections examined at various magnifications was made for each historic group and the relative degrees of microstructural defects were compared to the known prevalence of tooth decay during the various periods under study. However, the microstructure of prehistoric teeth compared to successive samples up to the time of Christ did not show statistically significant differences. The teeth from Archaic-Classic to the Romaic period, an example of which is shown in Figure 22, were not materially worse than the prehistoric period, but far better than those from contemporary Greece, Figure 23. Therefore, while the teeth of the Greek population showed evidence of developmental defects as far back as 3000 B.C., there appears to have been an increase in these defects in the course of recent history only.

### *Caries*

Dental decay was found in teeth representing all historic groups studied. Of the 40 prehistoric teeth, 6 showed carious lesions, 4 of which were in the cementum at the junction of the enamel. Of the 12 teeth from the Archaic-Classic period, 3 showed caries, all being lesions in occlusal fissures. Of the 7 teeth from the Hellenistic-Romaic period, one tooth, a first permanent molar, showed caries. Of the 4 teeth from the Posterior age, 2 showed caries, one a first permanent molar, the other a first premolar, and both were interproximal lesions. In the 23 teeth from New time, 8 showed caries, 4 of which were interproximal lesions, 3 cemental caries, and one occlusal fissural caries. The teeth from Present time were extracted from the mouths of children living in Greece today and were generally carious and in many cases severely decayed with lesions covering more than one tooth surface.

### Ancient Iceland

From the Stephanson collection of Old Icelandic skeletal remains donated to Harvard's Peabody Museum in 1905, 2 specimens were obtained through the courtesy of Professor Hooton. One was a maxillary left first permanent molar from a girl approximately 18 years old,

whose remains were found at Hoffjarthar, Iceland. The roots were intact; the crown showed little attrition and no caries. There were a few pronounced grooves in the occlusal fissure, but the texture of the enamel generally was smooth, and the color white with no staining. The other tooth was also a first permanent molar from an individual about 20 years of age, originating from Alptones, Iceland. All the cusps of the tooth were worn almost through the enamel. The surface of the tooth was quite smooth.

Histologically, these teeth showed no severe abnormalities in development. The dentin had a generally granular appearance (Fig. 24), areolar in spots, but only 3 interglobular dentin spaces were seen in the section of one tooth, 5 in the other. Except for a few slightly pronounced growth rings, the enamel appeared quite regular both in prism arrangement and in incremental pattern.

### Middle Age Norway

Teeth from Middle Age Norway were obtained from the Department of Anatomy, University of Oslo, through the courtesy of Professor K. E. Schreiner, Director of its Anthropological Collections. From skulls excavated in and near the French Maria Church in Gamlebyen, Oslo, in 1935, 23 teeth were selected by Dr. Schreiner and sent to this laboratory for microscopic examination. Of this sample, 3 were deciduous teeth: one incisor, one canine, and one molar; 20 were permanent teeth: 4 molars, 5 premolars, 5 maxillary incisors, 4 mandibular incisors, and 2 canines, one from each jaw. Most of the teeth had a well preserved white and glossy enamel surface, but many of the roots appeared dry and brittle, suggestive of post-mortem changes as described in a previous paper of this series.[4] Of the 23 teeth, the surface texture could be graded as normal for 8 teeth, including the 3 deciduous teeth, and grade I hypoplasia for 12, a moderate degree of hypoplasia; whereas 3 had gross hypoplastic defects in limited regions of the crowns. Two carious lesions were noted, one on the distal surface of a premolar, the other on the cementum of a third molar.

Microscopic examination of ground sections of the teeth revealed a somewhat higher incidence of developmental defects than could have been predicted by surface inspection. In the enamel, several pronounced incremental lines were noted in 7 of the teeth, whereas 9 teeth showed minor irregularities in the growth rings and isolated areas of pigmentation.

Four teeth exhibited regions in the dentin of numerous interglobular spaces, even though relatively minute in size (Fig. 25). Nine teeth

showed moderate evidence of these defects, and none were entirely free from some granularity in the dentin structure. One of the teeth with gross hypoplasia, in the form of a circular enamel groove, showed the presence of several quite pronounced incremental lines in the enamel when seen in section (Fig. 25), suggesting a distinct arrest in development.

In addition to these older teeth, a somewhat greater material of contemporary origin has been obtained from Norway and is being subjected to study by a similar sectioning method. At the moment it may be stated that more severe developmental defects in the enamel and dentin are very frequently encountered in these teeth, in the form of distinct interglobular spaces in the dentin and pigmentations and irregular incremental patterns in the enamel.

## Central American Indians

### *Guatemalan Indian Teeth*

Harvard's Peabody Museum, through the courtesy of Professor Hooton, made available 11 tooth specimens from Dr. Kidder's collection of skeletal remains found at Kamerol-Juym, Guatemala, in 1936 (Peabody no. N-6896). All of the teeth were from Mound A-1: 3 from Tomb no. 4, skeleton no. 3 of a child of about 10 years; 4 teeth from Tomb no. 6, skeleton no. 4; the remaining were isolated specimens. One of the teeth was a second deciduous molar; all the others were permanent teeth: 2 incisors, 1 canine, 4 premolars, and 3 molars. Due to post-mortem changes,[4] which were very marked in this sample, 3 teeth were disintegrated to such an extent that only the shells of the enamel remained, and these showed brown secondary discoloration. In the other teeth the roots had been either partly resorbed, as was true of the deciduous tooth, or partially lost due to post-mortem changes. Due to post-mortem changes in these teeth, it was impossible to make any reliable gradation of the original surface texture. With regard to histologic structure, there were marked developmental defects of the dentin in all of the teeth, especially those from the child (skeleton no. 3); from 8 to 25 interglobular dentin spaces could be demonstrated within comparably magnified areas. The enamel showed many areas of pigmentation, some of which may have been secondary, whereas frequent irregularities in the incremental pattern were definitely of developmental origin (Fig. 26) and were demonstrated within comparably magnified areas. This group of teeth appeared to be somewhat more defectively formed than teeth originating from other locations during comparable periods of time.

*Pecos Indian Teeth*

The Pecos Indian material originated from the ruins of the Indian Pueblo of Pecos, situated in the valley of the Pecos River in North Central New Mexico. From 1915 on, a total of 1,823 graves had been excavated. From the excavations by the Andover Expedition, 1929, the Peabody Museum has a collection of 973 Pecos Indian skeletons and thousands of isolated teeth.

The settlement from which this material was obtained is presumed to have existed from about 1100 to 1700 A.D. According to Hooton,[8] the skeletal remains were on the whole well preserved but not as well as those from arid, sandy countries, such as Egypt. The skeletons were found mostly in red clay with a good deal of sand in some places. Skeletons found in hard, dry clay were well preserved; those resting directly on sandstone were decomposed, and those in sandier soil were friable and brittle. The gross characteristics of the teeth and dentitions have been described by Hooton,[8] Rihan,[9] and Nelson.[10] Periodontal disease was present in 72 per cent of the population with marked pyorrhea in 13 per cent. Marked attrition of the teeth was present in 97 per cent of the people. Yet caries was most frequent in the occlusal surfaces with interproximal caries in about 30 per cent of the affected teeth. Twelve per cent of the children had caries in their deciduous teeth.

Altogether, about 50 per cent of the Pecos Indians had dental caries, and the teeth appeared to deteriorate more and more through the archeologic period studied. The inferiority of the teeth is believed to have been due to a diet of maize and vegetables, and it is fairly certain that the Pecos Indians were in most cases undernourished. In addition, the skeletal remains revealed that about 13 per cent of the Pecos Indians suffered from arthritic infection. About 2.5 per cent showed evidence of periostitis and osteomylitis of long bones and 3 per cent, of osteoporosis. Again there was evidence of an increase in these conditions in the later period of the settlement.

It was possible to examine a relatively much larger sample of teeth from Pecos Indians. A total of 1,218 isolated tooth specimens were first examined grossly with respect to the surface texture of the enamel. Of these, about 25 per cent appeared to have a perfectly smooth surface texture, whereas the largest number of teeth fell in the category of mild to moderate hypoplasia. From this over-all collection, Professor Hooton made available a random sample of 76 assorted teeth for further microscopic study, 69 permanent and 7 deciduous teeth. A relatively large proportion of the permanent teeth, 11 in all, were decayed.

Judged by binocular examination, the surface texture of the enamel

was as follows: 9 of the teeth could be graded as normal or perfect, 26 as M-hypoplasia grade I, 24 as grade II, 5 as grade III, and one as gross hypoplasia. The 11 carious teeth fell in the category of grades I and II hypoplasia.

The internal microstructure of these teeth was observed in histologic sections, mainly in ground sections. A great many sections showed post-mortem damage.[4] About one fourth of the material showed marked irregularities in the incremental pattern of the enamel, and few teeth were free from pigmentation and one or more pronounced growth rings. From 1 to 40 interglobular dentin spaces were found within comparably magnified areas within the crown portion of the teeth, and many of the teeth showed extremely poor calcification. To illustrate how severely affected some of these teeth were, Figure 27 shows a section of a maxillary central incisor in which the dentin is almost falling apart because of the great number of interglobular dentin spaces between incompletely fused globules. It will also be noticed that the dentin-enamel junction shows extreme scalloping, a condition which has been found in a number of the Pecos Indian teeth and may possibly have functional rather than pathologic significance.

## Discussion

Were one to draw any conclusions at all from the present study, which admittedly suffers from the unavoidable quantitative limitations of anthropologic material, it would be necessary to "pyramid one's assets," as Professor Sherwood L. Washburn put it in an address before a recent conference on bones and teeth.* If the perfect microstructure of wild rhesus monkeys (Figs. 3 and 4) is used as a normal baseline, then it would appear, by contrast, that what is the rule in this lower primate must have been an exception in human beings since the time of cave man. Faulty microstructure was nearly universal within our sample of human teeth originating from prehistoric, ancient, and recent periods of history and from widely separated parts of the world. With reference to anthropologic and especially pathologic features, some additional comments may be made.

Archeologic and paleontologic evidence suggests that the Palestinian Stone Age people from the Skhūl cave were antecedent to the various fragmentary individuals recovered from layer B of the Tabūn cave. Yet the specimens from both sites are regarded as members of the same species or race. Not only did they live in the same locality at approximately the same time, but their cultures seem to be almost

* The seventh Ross Pediatric Conference held under the auspices of Forsyth Dental Infirmary and Harvard School of Dental Medicine in Boston, Massachusetts, March, 1955.

identical. Anthropologic observations on the gross characters of their teeth are uniform, but McCown and Keith[5] pointed to a certain variability within this material, so much so, that if the findings had been made in separate and diverse localities, one might have been tempted to consider the various skeletons as from different forms of humanity. They suggest that the Mount Carmel people were in the process of an evolutionary change and, hence, that the variability must have been due to an evolutionary divergence. It is believed that the Krapina people come nearest to bridging the gap between the ancient Palestinians found at Mount Carmel and the Neanderthalians of western Europe. Of the Neanthrophic types, the Cromagnon man of southern France appears to come closest to the Skhūl people. The Tabūn people appear nearest to the people of Krapina, which incidentally suffered from fairly extensive tooth decay. The Mount Carmel people may represent not the ancestors of the Cromagnons but instead the Neanderthaloid collaterals or the cousins of the ancestors to the Cromagnons. In a recent study on the place of Neanderthal man in human evolution, Howell[11] suggested, on the basis of evidence now at hand, that modern man was a late arrival in the Pleistocene age and not of as great antiquity as has been believed in the past. He reached the conclusion that the *classic* Neanderthals, restricted to western Europe, were a peripheral development, that the *early* Neanderthals resemble modern man, and that the Stone Age people of Mount Carmel, Palestine, may be regarded as ancestral to modern man. These conclusions were based on morphologic, spatial, and temporal evidence obtained from a review of data in the literature.

From the standpoint of dental paleopathology, I have noted a certain degree of variability, but both the Skhūl teeth and Tabūn teeth, whether from Paleolithic or superficial layers, could well be grouped with those of modern man with respect to the range and severity of developmental defects in the dental microstructure. One possible cause of the faulty calcification of these teeth may have been primary inadequacies or irregularities in the calcifying property of the diet. Certainly the dietary habits of these cave dwellers must have been greatly different from the subhuman primates living in the tree tops or roaming in the woods, incapable of using tools other than their own teeth. For it is emphasized by McCown and Keith[5] that while the teeth of the Palestinian Stone Age people showed varying degrees of wear, none of them manifested the degree of attrition often met with in the dentition of modern native peoples, such as the Eskimos. It is true that there was generally a high rate of mortality among the races of the Pleistocene period, and McCown and Keith estimated that of

the Mount Carmel people, whose age at death could be determined, 4 died in childhood between 4 and 10 years, one was under 30, 5 between 30 and 40, one over 40 but under 50, whereas only one was over 50 years of age at the time of death. But even when age is taken into account, the teeth of these people were remarkably little worn, particularly those of the Skhūl men. Their diet must have been soft.

In addition to primary dietary inadequacies, it has been suspected for a long time that faulty calcification of the teeth may be secondary to other ailments. Before the turn of the century, Bödecker[12] suggested that interglobular dentin resulted from a temporary interruption of calcification owing to transient ailments of the mother. At the same time, Walkhoff[13] appears to have been the first to anticipate that various children's diseases indirectly may bring about failures in the calcification of enamel and dentin, possibly through secondary digestive disturbances.

It is noteworthy that the Stone Age people of Palestine appeared essentially free from decay despite the inferior developmental quality of their teeth. Yet one wonders whether they might not, in fact, have proved to be very *susceptible* to tooth decay, if these people suddenly had been exposed to the oral environment characteristic of modern man. In this connection it is difficult to improve upon the reasoning of M'Quillen,[14] who, in 1866, was one of the first to note the frequent distribution of interglobular dentin in human teeth extracted in his Boston practice. He was apparently the first to recognize that this condition, which the earliest observers had considered of significance to the metabolism of *normal* dentin, was probably an abnormality in development, predisposing such teeth to decay. Furthermore, he openmindedly made the important qualification, which has often been lost sight of, that "if protected from the action of external influences, or the *exciting causes of decay*, this *predisposition* might remain dormant for a lifetime." During their short life span, the Stone Age people of Mount Carmel appear to have been so protected.

A more recent example of such protection, in an isolated primitive-living community, has been observed on the island of Tristan da Cunha, whose inhabitants are famous for their freedom from decay and yet were found to suffer from comparable developmental microscopic defects in their teeth.[15] That absence of caries does not necessarily mean inherent *resistance* to caries is also evident from recent experimental observations in laboratory animals,[16] which have shown that the presence of caries is dependent upon the balance between a variety of modifying factors of genetic, developmental, hormonal, salivary, and bacterial, as well as of dietary nature. Therefore, my

observations do not necessarily contradict the concept of Mellanby[17] that the high susceptibility to caries in modern man may in part be related to inadequacies in the calcifying property of the diet during the period of tooth development. It may well be that civilized man, in order to withstand the impact of the oral environmental conditions prevailing today, would need teeth of developmental qualities superior to those of his ancestors, both from microstructural and microchemical points of view.

The fact that this approach to the caries problem has met relatively limited enthusiasm must undoubtedly be attributed to the impact of the bacteriologic concept of disease. Out of this approach grew Miller's so-called chemicoparasitic caries theory,[18] according to which the increase in caries in civilized man has been attributed largely to the external environment of the teeth. Nevertheless, it is interesting to note, in retrospect, that the developmental approach was anticipated before the turn of the century. Walkhoff, already cited,[13] in discussing the frequent occurrence of interglobular dentin spaces in contemporary human teeth concluded: (author's translation) a true prophylaxis and hygiene of the teeth . . . can come about only when the developmental defects of the tooth structure with regard to their origin, nature, and general importance become known to the medical world as well as the public at large. The road towards reduction of dental caries can be reached only when the predisposition of the teeth of civilized nations to caries can be changed . . . the predisposition has its main cause in the developmental faults of the hard tooth structure.

The exact nature of the mechanism of calcification and its failure in various deficiency states are still incompletely understood.[19] Primary nutritional deficiencies such as rickets, which has existed since ancient times,[20] may in part explain the prevalence of faulty calcification of human teeth. Further studies are needed to correlate the quality of calcification of teeth and bones in animals and man, primitive and modern. Thirty years ago, when Dickmann[21] reviewed and re-emphasized the probable importance of developmental dental defects in the etiology of caries, he suggested that it would be essential in this connection to undertake a thorough investigation of the teeth from monkeys and anthropoids as well as early man. But very limited material has been studied since then,[22] and a thorough comparative investigation of these developmental defects still remains to be made. My exploratory observations suggest that if the rhesus monkey is used as a standard of perfection, the teeth of early man were by comparison very prone to faulty tooth development; and other studies,[23] to be

published in this series, have revealed that subhuman anthropoids (wild chimpanzees, gorillas, gibbons, and orangutangs) tend to have teeth of an intermediary microstructure, less perfect than the monkey but superior to early man.

In pyramiding these limited assets, it is tempting to suggest in conclusion that the primates maintained uniformly perfect tooth structure only as long as they were basking in the sun of the tree tops, depending for survival upon the perfection of their teeth; that the terrestrial apes, roaming in the shadows of the trees and experimenting with newer ways of life, were able to perpetuate themselves despite occasional minor dental defects; and that early man proved too clever for his own good, dentally speaking, when he entered the cave and in the struggle for life discovered the use of other tools than his own teeth for the capture and preparation of food.

There are two principal attitudes toward this dentally discouraging course of human evolution. One was expressed in a monograph on the natural history of human teeth written by Murphy[24] in 1811. He conjectured that the susceptibility to caries originated during the time of tooth formation from constitutional disease which prevented the teeth from attaining "the degree of perfection, necessary to give them length of existance[sic]." However, when he noted the spread of dental disease following in the path of civilization, he merely reconciled himself to the fact that "men in a state of nature . . . are compensated by health, vigour, great animal perfection, and liberty, for the privation of the mental enjoyments, which, in civilization, arise from the study of philosophy and the sciences."

A more realistic attitude, commensurate with current potentialities of the sciences, would seem to lie in the encouragement of research directed toward a more precise identification of what constitutes perfectly developed teeth and toward the eventual duplication in human beings of such teeth. In future generations it may not only become feasible to prevent the severe microstructural defects in dental development reported in the present study, but also to incorporate into the teeth the optimal chemical ingredients in proper amount and ratio to each other. An important clue to the potentialities of this developmental approach stems from the peculiar dental significance of fluorine[25] and other trace factors.[26,27] Much more research is required along these lines; but, other things being equal, such as the oral flora and food substrate, it would appear that the susceptibility to dental caries may be modified to a significant degree by nutritional and other metabolic factors operating during the period of tooth development.[28]

## Summary

A study has been made of the microscopic structure of human teeth originating from Paleolithic Palestine, predynastic Egypt, prehistoric to recent Greece, Old Iceland, Middle Age Norway, and ancient to recent Guatemalan and Pecos Indians.

In an attempt to grade the degree of developmental defects in these teeth, observations have been made regarding deviations in the incremental pattern of enamel, and poorly calcified dentin has been graded in comparably magnified areas by counting the number of interglobular dentin spaces. It has been observed that human teeth from prehistoric and ancient periods, and from different locations of the world, exhibit developmental microscopic defects to a far greater extent than do the wild subhuman primates.


This investigation has extended over a period of nearly 10 years, during which support has been received in part through several annual grants from the Division of Research Grants and Fellowships of the United States Public Health Service, and in part from the Eugene Higgins' trust, Harvard University. For their generous cooperation in providing valuable specimens for this study, I am greatly indebted to Professor Earnest Hooton, Harvard University; Professor John Koumaris, University of Athens; and Professor K. E. Schreiner, University of Oslo. The skilled technical assistance of Mr. George Pettengill is also acknowleged.

## REFERENCES


1. Czermák, J. Beiträge zur mikroskopischen Anatomie der menschlichen Zähne. *Ztschr. f. Zool.*, 1850, **2**, 295–322.
2. Mellanby, M. Diet and the teeth: an experimental study. Part I, dental structure in dogs. *Medical Research Council, Special Report Series*, No. 140, His Majesty's Stationery Office, London, 1929.
3. Sognnaes, R. F. Preparation of thin "serial" ground sections of whole teeth and jaws and other highly calcified and brittle structures. *Anat. Rec.*, 1947, **99**, 133–144.
4. Sognnaes, R. F. Postmortem microscopic defects in the teeth of ancient man. *A. M. A. Arch. Path.*, 1955, **59**, 559–570.
5. McCown, T. D., and Keith, A. L. The Stone Age of Mount Carmel. The Fossil Human Remains from the Levalloiso-Mousterian. The Clarendon Press, Oxford, 1939, **2**, 390 pp.
6. Clark, W. E. L. The Fossil Evidence for Human Evolution; an Introduction to the Study of Paleoanthropology. The University of Chicago Press, Chicago, 1955, 180 pp.
7. Krikos, A. The progress of decay in Greece from the most ancient times down to the present. *Tr. Am. Dental Soc. of Europe*, 1935, 174–188.
8. Hooton, E. A. The Indians of Pecos Pueblo, a Study of their Skeletal Remains. Yale University Press, New Haven, 1930, 391 pp.
9. Rihan, H. Y. Dental and orthodontic observations on 289 adult and 53 immature skulls from Pecos, New Mexico. *Internat. J. Orthodontia*, 1932, **18**, 708–712.
10. Nelson, C. T. The teeth of the Indians of Pecos Pueblo. *Am. J. Phys. Anthropol.*, 1937–38, **23**, 261–293.

11. Howell, F. C. The place of Neanderthal man in human evolution. *Am. J. Phys. Anthropol.*, 1951, n. s. 9, 379–416.

12. Bödecker, C. F. W. The Anatomy and Pathology of the Teeth. S. S. White Dental Manufacturing Co., Philadelphia, 1894, 676 pp.

13. Walkhoff, O. Ueber das Wesen und die Entstehung von Entwicklungsfehlern in der Structur menschlicher Zähne und ihre Bedeutung für das spätere Leben. *Deutsche Monatsschr. f. Zahnh.*, 1895, 13, 305–319.

14. M'Quillen, J. H. The interglobular spaces in dentin. (An oral communication to the American Dental Association at the sixth annual meeting, held in Boston, August 1, 1866.) *Dental Cosmos*, 1866–67, 8, 113–118.

15. Sognnaes, R. F. Oral Health Survey of Tristan da Cunha. Det Norske Videnskaps-Akademi, Oslo, 1954, 145 pp.

16. Sognnaes, R. F. (ed.). Advances in Experimental Caries Research. American Association for the Advancement of Science, Washington, D.C., 1955, 236 pp.

17. Mellanby, M. Diet and the teeth: an experimental study. Part III, the effect of diet on dental structure and disease in man. *Medical Research Council, Special Report Series*, No. 191, His Majesty's Stationery Office, London, 1934.

18. Miller, W. D. Die Mikroorganismen der Mundhöle. Die örtlichen und allgemeinen Erkrankungen, welche durch dieselben hervorgerufen werden. G. Thieme, Leipzig, 1889, 305 pp.

19. Sognnaes, R. F. Microstructure and histochemical characteristics of the mineralized tissues. *Ann. New York Acad. Sc.*, 1955, 60, 545–572.

20. Ruffer, M. A. Studies in the Palaeopathology of Egypt. University of Chicago Press, Chicago, 1921, 372 pp.

21. Dickmann, E. Ein Beitrag zur Kenntnis von den sogenannten "Interglobularräumen" im menschlichen und tierischen Zahn. *Deutsche Monatsschr. f. Zahnh.*, 1925, 43, 653–672.

22. Erva, F. Untersuchungen über das sogenannte Interglobulardentin und die Tomes'sche Körnerschicht. Annales Academiae Scientiarium Fennicae, Vol. 40, Series A, No. 1, 195 pp., Helsinki, 1934.

23. Schuman, L., and Sognnaes, R. F. Developmental microscopic defects in the teeth of subhuman primates. *Am. J. Phys. Anthropol.* (In press.)

24. Murphy, J. L. A Natural History of the Human Teeth. J. Callow, London, 1811, 160 pp.

25. Shaw, J. H. (ed.). Fluoridation as a Public Health Measure. American Association for the Advancement of Science, Washington, D.C., 1954, 232 pp.

26. Constant, M. A.; Sievert, H. W.; Phillips, P. H., and Elvehjem, C. A. Dental caries in the cotton rat. XV. The effect of tooth maturity and minerals on caries production by semi-synthetic diets. *J. Nutrition*, 1954, 53, 29–41.

27. Sognnaes, R. F., and Shaw, J. H. Experimental rat caries. IV. Effect of a natural salt mixture on the caries-conduciveness of an otherwise purified diet. *J. Nutrition*, 1954, 53, 195–206.

28. Sognnaes, R. F. Is the susceptibility to dental caries influenced by factors operating during the period of tooth development? *J. Calif. State Dental A.*, 1950, 26, Suppl. 3, 37–52.

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[ *Illustrations follow* ]

## LEGENDS FOR FIGURES

FIGS. 1 and 2. Reproductions from original drawings by Czermák,[1] who in 1850 was the first to demonstrate the presence in human teeth of interglobular dentin spaces in which the calcifying globules of dentin have failed to fuse.

FIGS. 3 and 4. Ground sections of an incisor tooth from a wild rhesus monkey illustrate the perfect formation and calcification of the tooth substance characteristic of this subhuman primate.

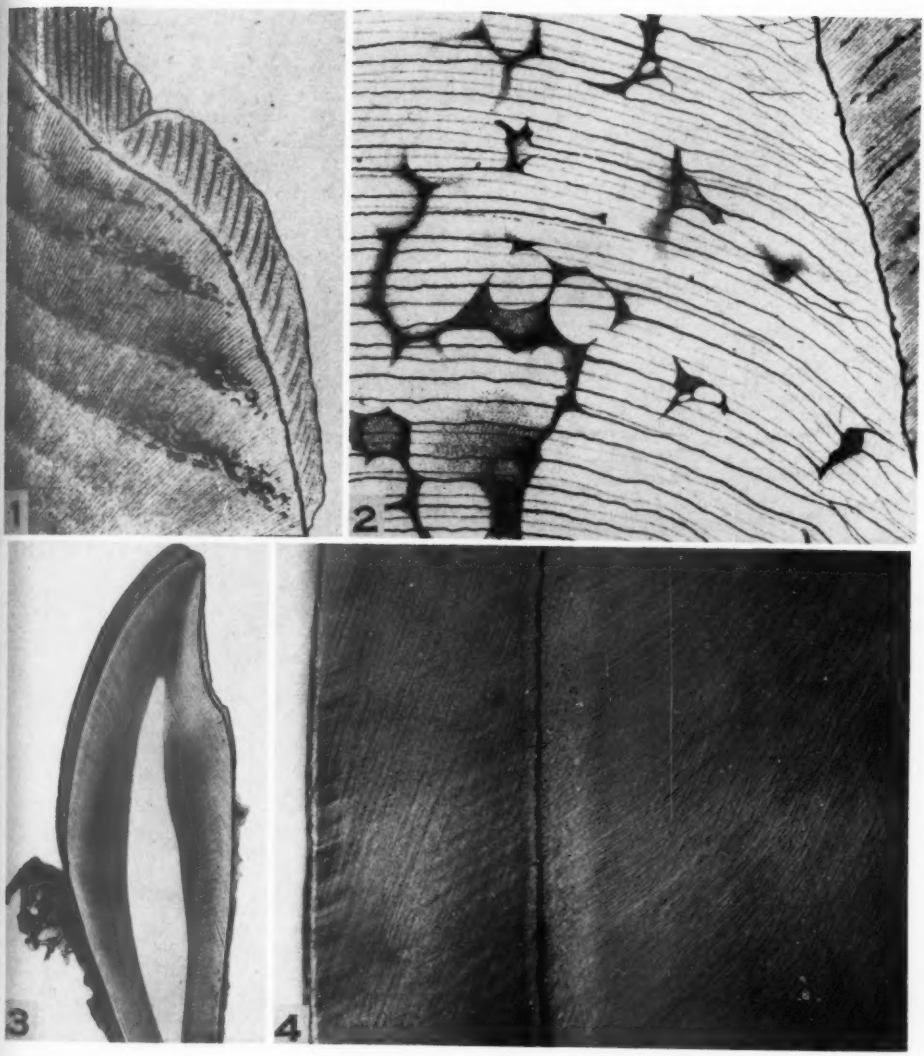
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FIG. 5. Ground section of mandibular right permanent second molar of adult female from the Skhūl cave of Mount Carmel, Middle-Paleolithic Palestine. In addition to post-mortem changes (dark patches), there is, on the distal surface, a cervical cavity involving enamel and dentin.

FIG. 6. Cervical region of the molar shown in Figure 5, suggesting a combination of caries and post-mortem erosion.

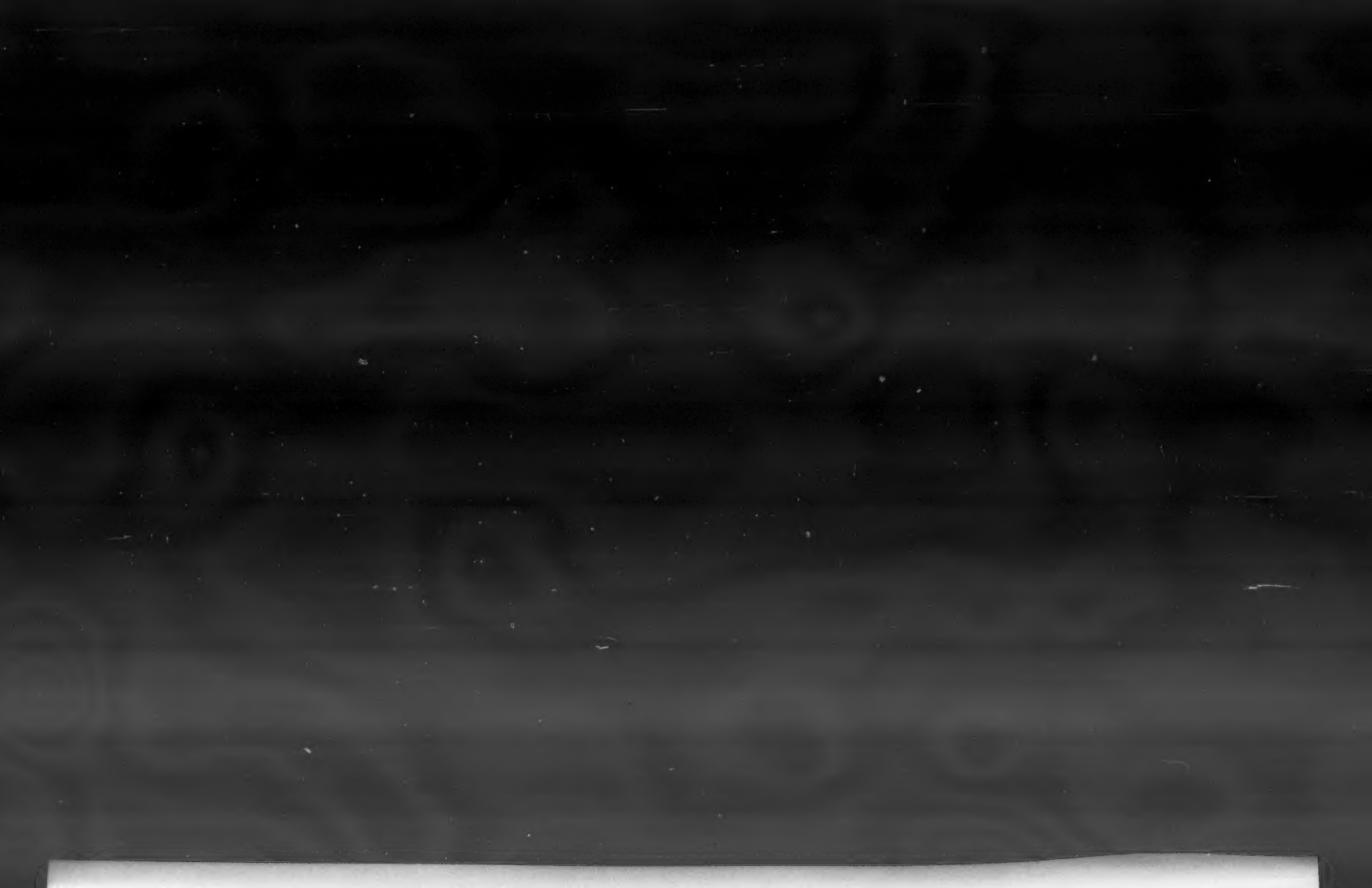
FIG. 7. Dentin and enamel from the same tooth as shown in Figure 5. In the coronal portion there is some irregularity in the distribution of the incremental lines of the enamel and considerable defects in the calcification of the dentin.

FIG. 8. Higher magnification of the dentin from the same tooth as shown in Figure 7, illustrating typical large interglobular dentin spaces.

FIG. 9. Enamel of maxillary right first permanent molar from Skhūl cave of Mount Carmel, Middle-Paleolithic Palestine. Several pronounced incremental lines are noted in the enamel, but the superficial dark areas are believed to be due to post-mortem erosion.

FIG. 10. Segment of dentin from the same tooth as shown in Figure 9. Failure in calcification is indicated by numerous interglobular dentin spaces.

FIGS. 11, 12, and 13. Maxillary right first permanent molar of an adolescent individual from the Tabūn cave, Mount Carmel, Middle-Paleolithic Palestine. There are pronounced incremental lines in enamel (Fig. 11), interglobular spaces in the dentin (Fig. 12), and between the two is a very scalloped dentin-enamel junction (Fig. 13).



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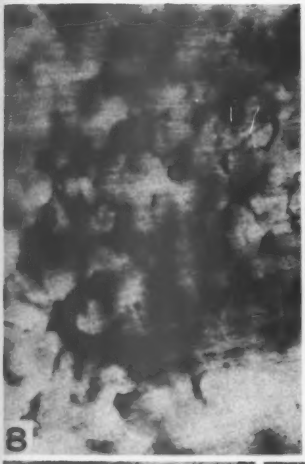
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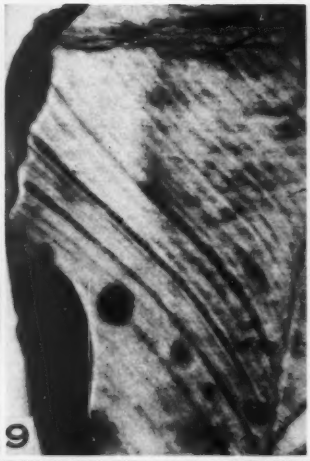
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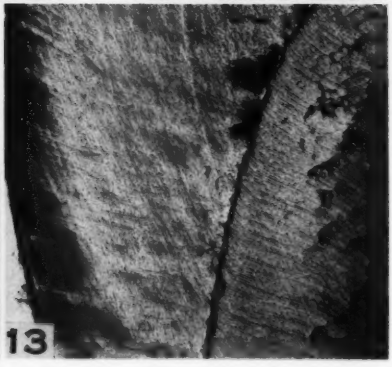
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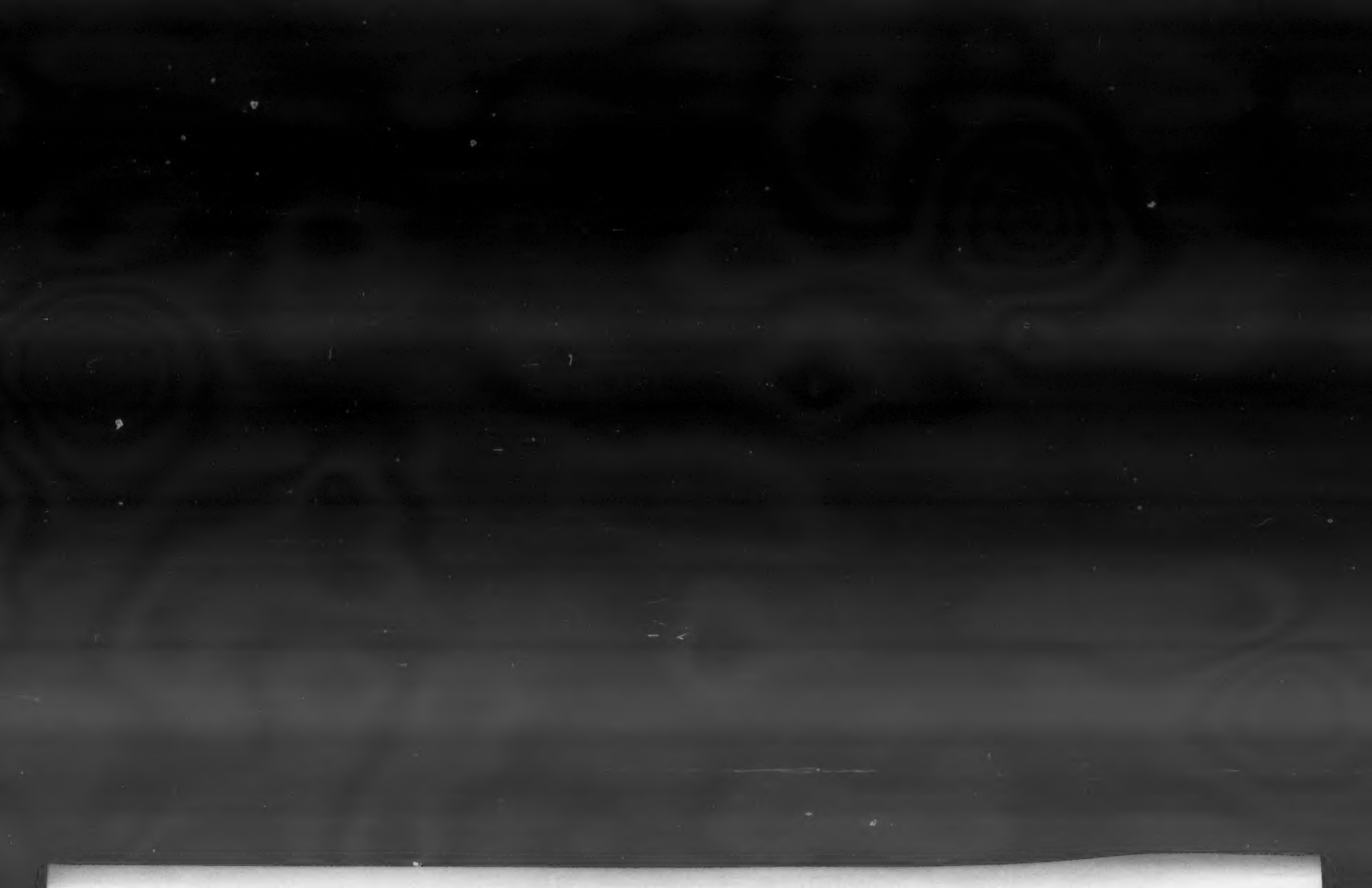
FIG. 14. Maxillary left central incisor originating from a young adult individual, in Tabūn's layer B, Mount Carmel, Middle-Paleolithic Palestine. Ground sections of this tooth showed a number of pronounced incremental lines, several Owen's contour lines in the dentin, and a general granular appearance of the dentin structure.

FIG. 15. Mandibular left first permanent molar of a young child from the Tabūn cave, Mount Carmel, Middle-Paleolithic Palestine, Miscellaneous Series III, layer B. Enamel tufts are well seen and one of the incremental lines near the surface is particularly noticeable.

FIGS. 16 to 18. Mandibular left canine of young adult from the Tabūn cave, Miscellaneous Series V, found in superficial deposits in front of the cave, probably not of paleoanthropic type. There are very pronounced incremental lines in the enamel (Fig. 16), a great many small interglobular spaces in the dentin (Fig. 17), and general granularity and irregularity of the tooth structure (Fig. 18).

FIG. 19. Maxillary right first permanent molar of older individual from Tabūn's superficial layers, Miscellaneous Series V. Very extensive defects are noted in the dentin in the form of interglobular dentin spaces and also some irregularity in enamel development.



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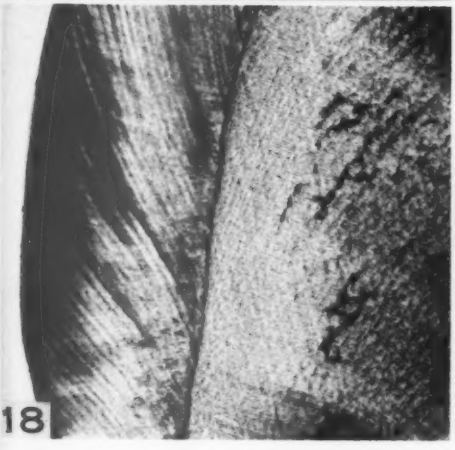
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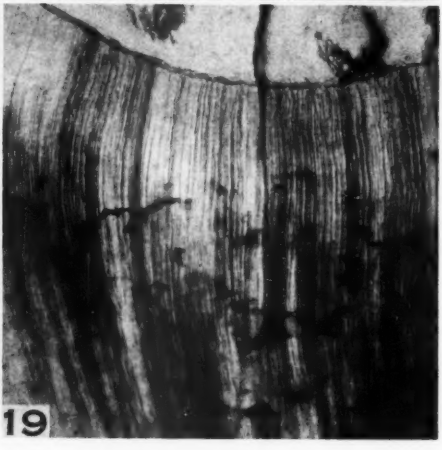
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FIG. 20. Maxillary right third permanent molar from predynastic Egypt. The dentin shows a general granularity in texture and also some interglobular dentin spaces.

FIG. 21. Mandibular first right permanent premolar from prehistoric Greece (Asine, Peloponnesus, Mycenae). The enamel shows a pronounced but comparatively regular incremental pattern, whereas the dentin has an areolar and granular calcification.

FIG. 22. Deciduous molar from Archaic-Classic Greece (Piraeus, Attica) showing two pronounced incremental lines in the enamel, the internal one presumably the neonatal line which by its location may suggest premature birth. The dentin is relatively normal with minor granularity.

FIG. 23. Deciduous molar from modern Greece, Thassos Island, 1948. There are very pronounced incremental lines in the enamel with areas of pigmentation. The dentin is extremely defective with numerous interglobular spaces.

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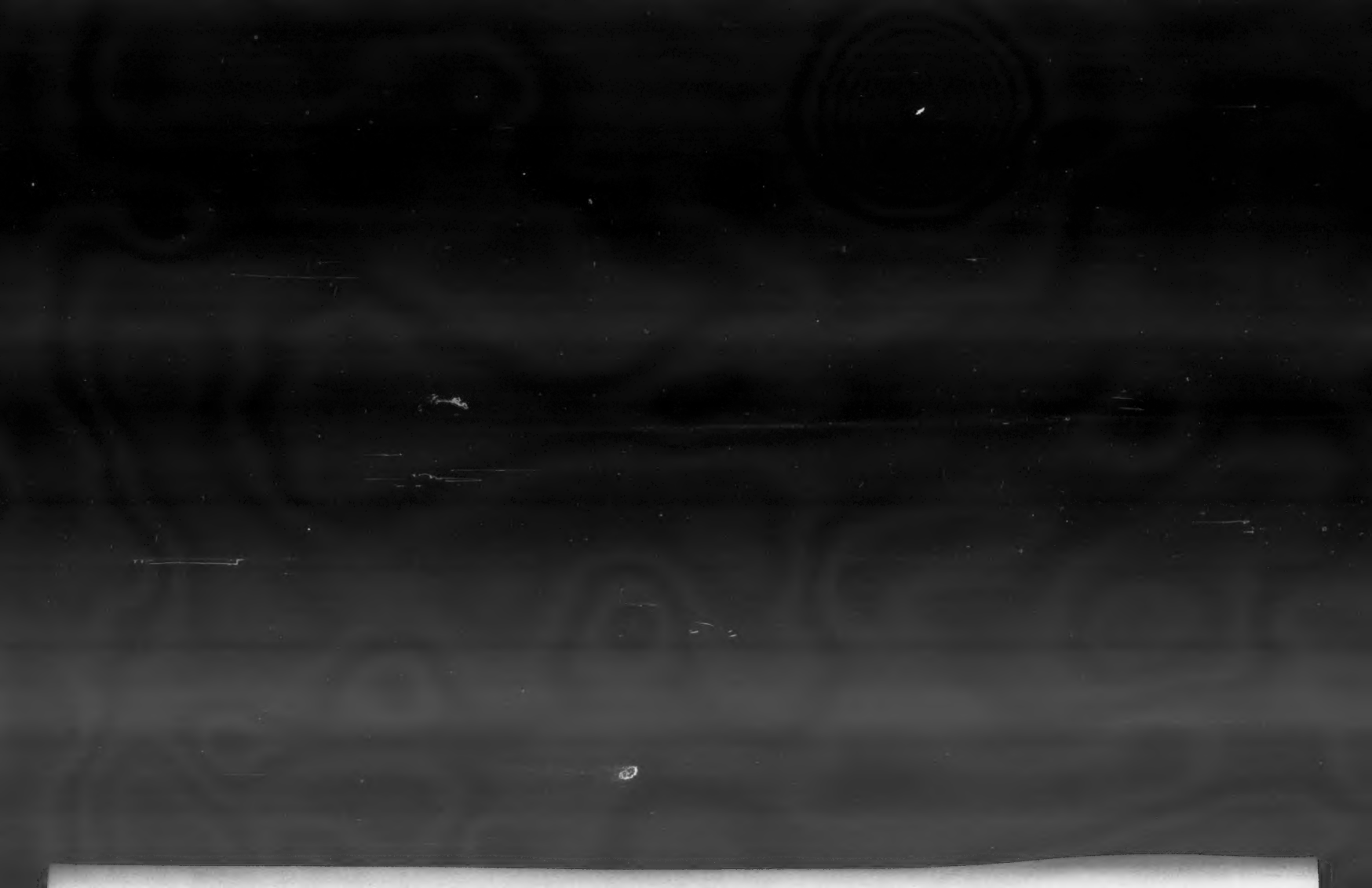
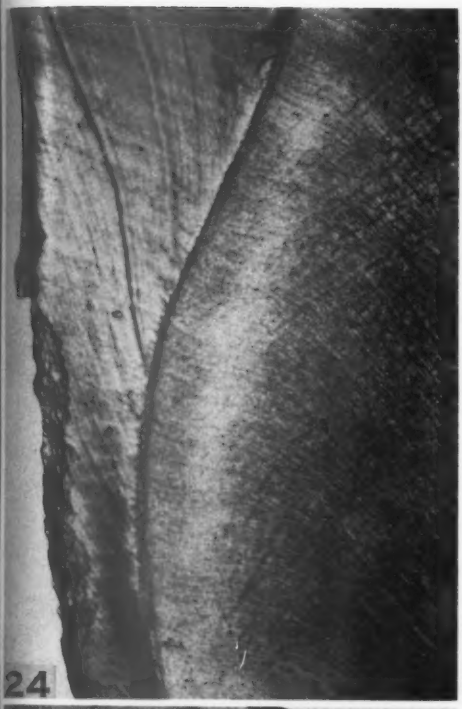
FIG. 24. Maxillary right first permanent molar of 18-year-old female from Old Icelandic period (Peabody no. 57884). There are several pronounced incremental lines in the enamel, and the dentin structure varies from granular to areolar with a few small interglobular dentin spaces.

FIG. 25. Maxillary left first permanent incisor from Middle Age Norway. The enamel shows evidence of hypoplasia with deviation in the incremental pattern. In the dentin there are numerous but relatively small interglobular dentin spaces.

FIG. 26. Molar tooth from Guatemalan Indian. Dentin shows granular character with some small interglobular dentin spaces. Several pronounced incremental lines are noted in the enamel.

FIG. 27. Molar from Pecos Indian with extremely defective calcification of dentin, showing not only interglobular dentin spaces but areas where individual globules are completely separated by uncalcified matrix. There is a very pronounced scalloping of the dentin-enamel junction.





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# THE CARCINOGENIC ACTION IN THE MOUSE OF 20-METHYLCHOLANTHRENE BY RECTAL ADMINISTRATION *

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Adenocarcinoma of the glandular mucosa of the mouse's stomach can be induced by the injection of polycyclic aromatic hydrocarbons into the wall of that organ,[1-4] but not when such carcinogens are administered by mouth.[5-8] One of the explanations put forward to account for this discrepancy is that the mucin secreted by the glandular mucosa serves as a physical barrier to the penetration of the water-insoluble hydrocarbon when acting from within the lumen.[6] This hypothesis was somewhat weakened by the finding that when such carcinogenic hydrocarbons are dissolved in hydrophilic-lipophilic solvents, they are able to penetrate the mucosa, as demonstrated by fluorescence microscopy,[9] but still fail to act carcinogenically on that tissue.[5,10] The squamous epithelium of the forestomach, on the other hand, is readily responsive to their action, whether they are dissolved in hydrophilic-lipophilic or in ordinary lipid solvents. The fact that the glandular mucosa of the stomach has a complex physiologic function, secreting HCl and pepsin, as well as mucin, is an added complication. An alternative method of inquiry would, therefore, be to test the action of carcinogenic hydrocarbons on some other mucin-secreting tissue, e.g., the rectum, in which these complicating factors are not involved.

The present investigation deals with the action of 20-methylcholanthrene on the rectum of the mouse, using three different methods of administration, two being concerned with its introduction into the lumen and one with its injection into the wall of that organ.

## METHODS

The animals used in these experiments were female Swiss mice, 5 months old, inbred in these laboratories by brother-to-sister mating for 8 or 9 generations since acquiring the strain (from a colony previously maintained by pen breeding). They were fed Purina Laboratory Chow and water *ad libitum*, and kept in an air-conditioned room at about 22° C.

* Received for publication, August 22, 1955.

The carcinogen used was 20-methylcholanthrene (m.p. corr., 176.2° to 177.1° C.) as a 0.5 per cent solution in undiluted polyethylene glycol-400 (Union Carbide and Carbon Corp., U.S.A.). This solution was administered to three groups of mice in the following ways.

(1) Short lengths of thin plugs of absorbent cotton (about 1 mm. in diameter and 1 cm. long) were soaked in the carcinogenic solution and introduced into the rectum, to about 2 cm. from the anal orifice. They remained there until the next evacuation of feces. Frequency of administration: twice weekly for the first 12 weeks, and once weekly thereafter. The insertion was made through a metal tube as a guide, to avoid contamination of the anal skin by the carcinogen.

(2) The solution of carcinogen (0.05 ml.) was introduced into the rectum by means of a syringe and a no. 20 blunt-ended needle, the tip being inserted to about 1.5 cm. from the anal orifice. Frequency of administration: as in (1).

(3) The carcinogenic solution (0.2 ml.) was administered as a single injection into the submucosa of the rectum, from inside the lumen, at a level of about 1 cm. from the anal orifice. These animals received no further treatment.

All mice that died during the course of the experiment were necropsied and the rectum and surrounding tissues were fixed in Zenker's and Bouin's solutions for histologic sections. The surviving animals were killed at the 37th week of the experiment and similarly examined. Sections were stained by Harris' hematoxylin and eosin and, in some cases, also by van Gieson's method and by Schiff's periodic stain.

## Experimental Findings

The earliest tumor to appear in any of the mice was after 12 weeks from the beginning of treatment. The incidence, expressed as percentage of *effective* total, is therefore calculated on the basis of animals that survived more than 12 weeks.

*Experiment I (30 Mice): Cotton Plugs Soaked in Methylcholanthrene Solution, Inserted into the Lumen of the Rectum.* There were 23 survivors in experiment I after 12 weeks of treatment. Of these 2 (9 per cent) developed tumors—all sarcomas—in the tissues surrounding the rectum. Their times of appearance, histologic types, probable sites of origin, and the organs invaded are summarized in Table I.

*Experiments II and III (20 and 30 Mice, Respectively): Solution of Methylcholanthrene Injected into the Lumen of the Rectum.* Eighteen of the 50 mice in experiments II and III died during the first 12 weeks. Of the remaining 32, 8 (25 per cent) developed tumors in the tissues surrounding the rectum. These were all sarcomas, except

that in one case there was, in addition, a squamous cell carcinoma, apparently derived from the anal skin (Table I).

*Experiment IV (30 Mice): Solution of Methylcholanthrene Injected into the Wall of the Rectum (Once Only).* Four of the 30 mice

TABLE I

*Tumors Developing in the Tissues Surrounding the Rectum Following the Administration of 20-Methylcholanthrene Introduced into the Lumen of the Rectum*

| Mouse no.* | Duration of treatment | Tumor type | Probable site of origin | Comments |
|---|---|---|---|---|
| | *wks.* | | | |
| R I–13 | 19 | Myosarcoma | Muscularis mucosae and/or muscularis | Invading serosa and uterus |
| R I–17 | 21 | Fibrosarcoma | Not ascertainable | |
| R II–14 | 18 | Myosarcoma | Muscularis | Invading serosa |
| R II–17 | 20 | Myosarcoma | Muscularis | Invading serosa, including ureter |
| R II–19 | 21 | Myosarcoma | Muscularis | |
| R III–15 | 15 | Mixed cell sarcoma | Not ascertainable | |
| R III–17 | 18 | Myosarcoma and squamous cell carcinoma | Muscularis and anal skin(?) | Invading serosa |
| R III–18 | 18 | Myosarcoma | Muscularis | Invading serosa and lymph node |
| R III–24 | 21 | Myosarcoma | Muscularis mucosae and/or muscularis | Invading serosa |
| R III–25 | 24 | Myosarcoma | Muscularis | Invading serosa |

* R I series = cotton plugs soaked in methylcholanthrene inserted into the lumen of the rectum. R II and R III series = methylcholanthrene solution injected into the lumen of the rectum.

in experiment IV died during the first 12 weeks. Of the remaining 26 mice, 13 (50 per cent) developed tumors in the tissues surrounding the rectum. All were sarcomas at the site of injection (Table II).

## HISTOLOGIC FINDINGS

Of the 23 tumors examined, 19 were judged, on the basis of histologic examination, to be leiomyosarcomas; 2, fibrosarcomas; 1, mixed cell sarcoma; and 1, a double tumor containing both myosarcoma and squamous cell carcinoma. Eighteen of the 23 tumors showed invasion of the serosa; 3, of the uterus; 3, of muscle; 3, of adipose tissue; 3, of lymph nodes; 1, of bone; and 1, invasion or inclusion of the ureter.

The histologic diagnosis of leiomyosarcoma was made both on the basis of the cellular structure of the tumor and its staining properties,

and on the recognition, in most cases, of evidence of origin of the tumor from the muscularis (stratum longitudinale of the muscular coat) or from the muscularis mucosae (Fig. 1). Most of the cells in these tumors were large and spindle-shaped, with abundant eosino-

TABLE II

*Tumors Developing in the Tissues Surrounding the Rectum Following the Administration of 20-Methylcholanthrene Injected into the Wall of the Rectum*

| Mouse no. | Duration of treatment | Tumor type | Probable site of origin | Comments |
|---|---|---|---|---|
| | wks. | | | |
| R IV–5 | 12 | Myosarcoma | Muscularis mucosae and/or muscularis | Invading serosa and uterus |
| R IV–6 | 12 | Myosarcoma | Muscularis mucosae and/or muscularis | Invading serosa and adipose tissue |
| R IV–7 | 13 | Myosarcoma | Muscularis mucosae and/or muscularis | Invading serosa and including uterus |
| R IV–8 | 13 | Fibrosarcoma | Not ascertainable | Invading muscle and adipose tissue (partly necrotic) |
| R IV–9 | 14 | Myosarcoma | Muscularis mucosae and/or muscularis | |
| R IV–10 | 14 | Myosarcoma | Muscularis | Invading serosa, muscle, and adipose tissue |
| R IV–12 | 14 | Myosarcoma | Muscularis | Invading serosa, muscle, and bone |
| R IV–13 | 14 | Myosarcoma | Muscularis | Invading serosa |
| R IV–14 | 15 | Myosarcoma | Muscularis mucosae and/or muscularis | Invading serosa |
| R IV–15 | 16 | Myosarcoma | Muscularis | Invading serosa and lymph node |
| R IV–16 | 18 | Myosarcoma | Muscularis | Invading serosa |
| R IV–17 | 18 | Myosarcoma | Muscularis | Invading serosa, lymph node, and muscle |
| R IV–18 | 20 | Myosarcoma | Muscularis | Invading serosa |

philic cytoplasm; among these were many giant cells (Fig. 2). In others, the cells showed pleomorphism, with round or oblong nuclei as the predominant form. In these, mitotic figures were particularly common. The cells tended to form interlacing bundles running in different directions; stroma was scarce; and vascular slits, bordered by tumor cells without any endothelial lining, were encountered frequently.

The two fibrosarcomas consisted of cells smaller than those seen in the leiomyosarcomas, more fusiform, and more uniform in size and shape, with scarce cytoplasm and fine fiber-like processes.

The double tumor (R III-17) contained, in addition to areas of typical myosarcoma, characteristic areas of squamous cell carcinoma, consisting of solid strands of polygonal cells, separated by intercellular stroma. In the centers of these areas, eosinophilic whorls of keratin were present (Fig. 3). Mitotic figures were numerous in both the sarcomatous and carcinomatous regions.

In no case was there any evidence of a tumor derived from the glandular mucosa of the rectum, nor were there any signs of what could even remotely be considered as a precancerous lesion of the glandular mucosa, though small ulcers with underlying inflammatory changes were observed in a few cases.

*Penetration of Carcinogenic Hydrocarbons into the Rectal Mucosa*

The penetration and distribution of carcinogenic hydrocarbons in living tissues can be studied conveniently by fluorescence microscopy of unstained frozen section after such tissues have been in contact with the carcinogen *in vivo* for varying intervals.[9,11,12] A number of such fluorescence microscopic examinations were undertaken in the course of the present investigation, both on gastric and rectal mucosa (i.e., after feeding and rectal injection, respectively), using solutions of 20-methylcholanthrene in polyethylene glycol-400 and also 3, 4-benzpyrene in the same solvent (the latter hydrocarbon having a more powerful fluorescence and being, therefore, more effective for such studies). The equipment used was a Hanovia U.V. lamp and a normal microscope fitted with a quartz condenser.

The conclusion of Ekwall *et al.*,[9] that such carcinogens penetrate the gastric glandular mucosa when polyethylene glycol was used as solvent, was confirmed, the fluorescence remaining visible for about 6 hours in the glandular mucosa and for about 36 hours in the squamous epithelium of the forestomach. In respect to the rectal mucosa, on the other hand, only a trace of diffuse fluorescence in the glandular mucosa could be detected ½ hour after the rectal injection, and none at all thereafter. It would seem, therefore, that in the rectal mucosa, the mucin secretion does act as a fairly effective physical barrier to the penetration of the carcinogenic hydrocarbon, even when a hydrophilic-lipophilic solvent is used as vehicle.

## Discussion

As mentioned at the outset, the rectal mucosa was chosen for this investigation because it represented a relatively simple mucin-secreting epithelium, in contrast to the rather complex gastric glandular mucosa. It was hoped that the results might throw light on the

unexplained phenomenon that carcinogenic hydrocarbons cause tumors to appear in the glandular mucosa of the mouse stomach when injected intramurally in that organ but not when administered by mouth.

From this point of view, the investigation proved unsuccessful, for reasons that could hardly have been predicted: (a) the rectal mucosa of the mouse does not respond to the carcinogenic action of 20-methylcholanthrene, *even when this is injected intramurally,* although sarcomas developed readily in the submucosa; (b) sarcomas developed also when the carcinogen was introduced only into the lumen of the rectum; and yet (c) diffusion of the carcinogen into the tissues was very slight, as demonstrated by fluorescence microscopy. Two conclusions may be drawn from these observations: (1) In mice, the rectal mucosa differs from that of the glandular portion of the stomach, in being unresponsive to methylcholanthrene carcinogenesis, even when the mucin barrier is side-stepped (by injecting the carcinogen into the subserosa). (2) The development of sarcomas, when the carcinogen is merely inserted into the lumen, is presumably due to some abnormal kind of penetration of the carcinogen, such as that which might occur when small ulcers, or other slight mechanical defects, develop during the course of frequently repeated injections.

The positive results of this investigation—the induction of sarcomas—confirm those of Van der Schueren and Vermeire,[13] who likewise obtained a leiomyosarcoma in a mouse following the introduction of methylcholanthrene into the lumen of the rectum. A similar result has been obtained in the rat.[14] The only examples of neoplastic lesions affecting the glandular mucosa in that region, through carcinogenic treatment, are those of Krebs[15] and of Lushbaugh and Hackett.[16] The former reported the development of an adenocarcinoma with squamous metaplasia at the junction of the colon and rectum, following repeated injections of 50 per cent alcohol per rectum.* The latter investigators described the development of an adenocarcinoma and other "adenomatous infiltrating lesions" of the colon, in rats fed a diet containing a motor lubricating oil.

The fact that so high a proportion of the sarcomas, in the present series, proved to be leiomyosarcomas—a type rarely found in man—is not out of keeping with the results of others[17] using mice as test animals. In guinea-pigs, on the other hand, liposarcoma—also rare in man—is a type commonly encountered following subcutaneous in-

* We have repeated the experiment on 20 Swiss mice, using the identical technique of Krebs.[15] The experiment was continued for 37 weeks, when 11 survivors were still present. All of the animals were examined histologically, and no tumor of the rectum or colon was found in any of them.

jection of carcinogenic hydrocarbons.[18] This illustrates once again the fact that different species, and sometimes even different strains of the same species, differ strikingly in the relative sensitiveness of their various tissues to spontaneous and induced tumor development.

## SUMMARY

After repeated injections of methylcholanthrene solution in polyethylene glycol-400 into the rectum of mice, 8 of 32 survivors developed sarcomas in the tissues of the submucosa. After repeated insertions of small cotton plugs, soaked in methylcholanthrene solution, into the rectum of mice, 2 of 23 survivors developed sarcomas similarly situated. After a single injection of the methylcholanthrene solution into the wall of the rectum, 13 of 26 survivors developed sarcomas at the sites of injection.

Of the 23 tumors thus produced, 19 were leiomyosarcomas, 2 were fibrosarcomas, 1 was a mixed cell sarcoma, and 1 consisted of both myosarcoma and squamous cell carcinoma, the latter apparently having origin in the anal epithelium. In none of the treated animals was there any evidence of a response on the part of the glandular mucosa of the rectum to the carcinogenic action.

Almost no penetration of the carcinogen into the rectal mucosa was demonstrated by fluorescence microscopy.

The results are of special significance with reference to the problem of gastric carcinogenesis, in which mucin secretion is also a factor.

## REFERENCES

1. Hare, W. V.; Stewart, H. L.; Bennett, J. G., and Lorenz, E. Tumors of the glandular stomach induced in rats by intramural injection of 20-methylcholanthrene. *J. Nat. Cancer Inst.*, 1951–52, **12**, 1019–1055.
2. Howes, E. L., and de Oliveira, J. R. Early changes in the experimentally produced adenomas and adenocarcinomas of the stomach. *Cancer Research*, 1948, **8**, 419–426.
3. Rusch, H. P.; Baumann, C. A., and Maison, G. L. Production of internal tumors with chemical carcinogens. *Arch. Path.*, 1940, **29**, 8–19.
4. Stewart, H. L., and Lorenz, E. Adenocarcinoma of the pyloric stomach and other gastric neoplasms in mice induced with carcinogenic hydrocarbons. *J. Nat. Cancer Inst.*, 1942–43, **3**, 175–189.
5. Berenblum, I., and Haran, N. The influence of dose of carcinogen, emptiness of stomach, and other factors on tumor induction in the forestomach of the mouse. *Cancer Research*, 1955, **15**, 504–509.
6. Ivy, A. C. Gastric physiology in relation to gastric cancer. *J. Nat. Cancer Inst.*, 1944–45, **5**, 313–330.
7. Klein, A. J., and Palmer, W. L. Experimental gastric carcinoma. A critical review with comments on the criteria of induced malignancy. *Arch. Path.*, 1940, **29**, 814–844.

8. Sugiura, K. The relation of diet to the development of gastric lesions in the rat. *Cancer Research*, 1942, **2**, 770–775.

9. Ekwall, P.; Ermala, P.; Setälä, K., and Sjöblom, L. Gastric absorption of 3,4-benzpyrene. II. The significance of the solvent for the penetration of 3,4-benzpyrene into the stomach wall. *Cancer Research*, 1951, **11**, 758–763.

10. Saxen, E.; Ekwall, P., and Setälä, K. Squamous cell carcinoma of the forestomach in mice following oral administration of 9,10-dimethyl-1,2-benzanthracene solubilized in aqueous solutions of association colloids. *Acta Unio Internat. Cancrum*, 1950–52, **7**, 156–159.

11. Berg, N. O. Comparison of the epidermal hyperplasia in the skin of mice after application of carcinogenic and noncarcinogenic irritants. *Acta path. et microbiol. Scandinav.*, 1948, **25**, 34–44.

12. Graffi, A. Zelluläre Speicherung cancerogener Kohlenwasserstoffe. *Ztschr. f. Krebsforsch.*, 1939–40, **49**, 477–495.

13. Van der Schueren, G., and Vermeire, J. Production expérimentale de leiomyosarcome du rectum et d'autres tumeurs chez la souris. *Rev. belge sc. méd.*, 1942, **14**, 42–47.

14. Laurens, J., and Bacon, H. E. Studies on experimental carcinogenesis in the colon and rectum of the rat. *J. Nat. Cancer Inst.*, 1951–52, **12**, 1237–1243.

15. Krebs, C. Experimenteller Alkoholkrebs bei weissen Mäusen. *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1928, **59**, 203–218.

16. Lushbaugh, C. C., and Hackett, A. An infiltrating adenomatous lesion of the colon of rats ingesting motor lubricating oil (S.G.F. No. 1 oil). *J. Nat. Cancer Inst.*, 1948–49, **9**, 159–172.

17. Shimkin, M. B., and Bryan, W. R. Morphology and growth of subcutaneous tumors induced with carcinogenic hydrocarbons in strain C3H male mice. *J. Nat. Cancer Inst.*, 1943–44, **4**, 25–35.

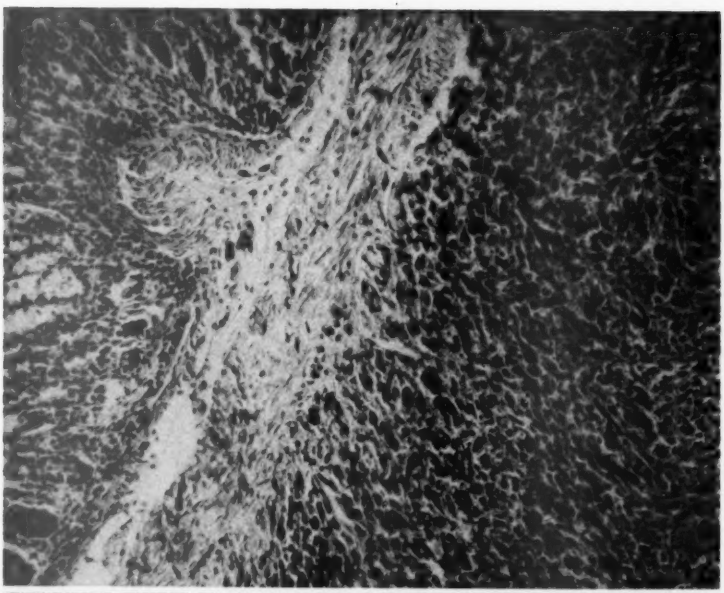
18. Shimkin, M. B., and Mider, G. B. Induction of tumors in guinea pigs with subcutaneously injected methylcholanthrene. *J. Nat. Cancer Inst.*, 1940–41, **1**, 707–725.

## LEGENDS FOR FIGURES

All sections were stained with hematoxylin and eosin.

FIG. 1. Mouse R III-25. Wall of rectum, with mucosa (left), muscularis, and tumor (right), showing the tumor arising from the outer muscular layer. The tumor shows pleomorphism with giant cells. × 150.

FIG. 2. Mouse R III-17. Leiomyosarcoma, showing spindle-shaped cells interspersed with giant cells. × 500.



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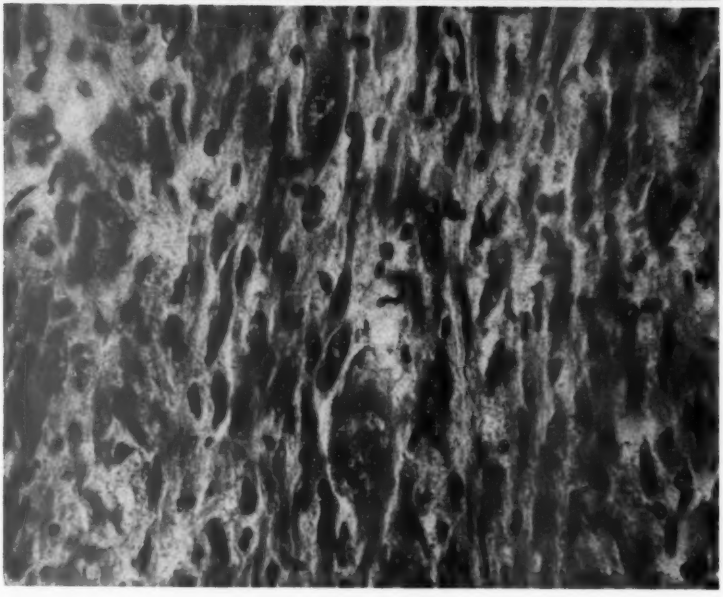

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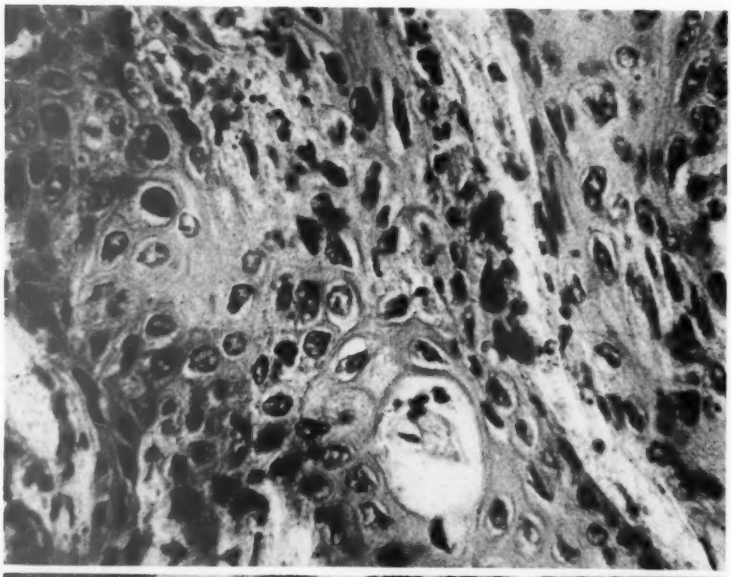
FIG. 3. Mouse R III-17. Squamous cell carcinoma with some cornification. This tumor was adjacent to the leiomyosarcoma shown in Figure 2. × 560.

FIG. 4. Mouse R IV-10. Myosarcoma, showing invasion of adipose tissue. × 500.



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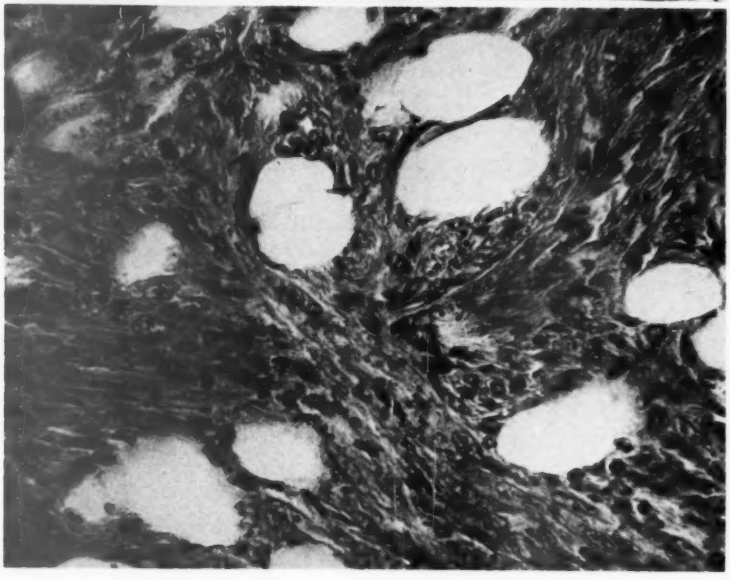

4

# CEROID IN FISH *

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Since the original description of ceroid in rats,[1,2] many papers have appeared on the etiology and characteristics of this pigment. It was first seen as a yellow, granular pigment in hematoxylin and eosin sections of the cirrhotic livers of choline deficient rats. The pigment was more fully characterized by Endicott and Lillie,[3] and additional staining reactions were summarized recently by Lillie.[4] The pigment is sudanophilic in paraffin sections, acid-fast, basophilic, isotropic, iron negative, and highly resistant to solution in water, alcohol, fat solvents, and dilute aqueous acids and alkalis. It is stained by Mallory's hemofuscin stain and Weigert's myelin stain. It reduces osmium tetroxide and diamine silver carbonate but not ferric ferricyanide. The Gmelin reaction for bile pigments is negative. It has a greenish yellow fluorescence at 3650–3660 Å. It is Schiff positive with or without antecedent diastase digestion after performic or periodic acid oxidation.

Ceroid originally was considered a characteristic and specific microscopic finding in experimental hepatic cirrhosis of dietary origin.[5] Later, however, it was established that dietary cirrhosis could be produced without ceroid and that cod-liver oil enhanced the formation of the pigment.[6] Endicott[7] reported that prolonged *in vitro* oxidation of cod-liver oil or linseed oil by potassium bichromate produced acid-fast material similar to ceroid. He noted that these oils, when injected subcutaneously into rats, gradually acquired acid-fastness. Victor and Pappenheimer[8] confirmed that dietary cirrhosis unaccompanied by pigmentary deposits could be produced. They noted that ceroid was not dependent either upon fatty changes in liver cells or upon cirrhosis and that vitamin E retarded the appearance of the pigment. They described ceroid for the first time in humans, in 4 cases "of profound nutritional disorder."[9] They also found acid-fast pigments in many routine human necropsy cases. Similar pigments were found in 55 per cent of 38 cases of Laennec's cirrhosis. From their observations and a study of the literature, they concluded that ceroid was found in association with: (1) vitamin E deficiency of rats and other laboratory animals; (2) dietary cirrhosis in rats, and other cirrhosis of rats and other laboratory animals; (3) chronic biliary

* Received for publication, July 8, 1955.

fistulae and experimental anemia in dogs; (4) nutritional disorders in man in which there was presumptive impairment of absorption by the small intestine; (5) cirrhosis of the liver and hemochromatosis in man; and (6) focal degenerative lesions. Mason *et al.*[10] reported on experiments with rats indicating that vitamin E, when added to diets rich in unsaturated glycerides, acted as an antioxidant, enabling the fat cells to incorporate and stabilize unsaturated fatty acids as "storage fat."

By 1950, ceroid had been described in rats by many investigators under a variety of experimental conditions usually involving low-protein, high-fat diets and cod-liver oil or a deficiency of vitamin E. It had been seen in vitamin E-deficient guinea pigs,[11] and in dogs and humans.[9] Edwards and Dalton[12] described the pigment in mice following treatment with carbon tetrachloride, but Lee[13] pointed out that the pigment observed in rats and in mice was not identical in every respect and implicated cellular necrosis as an important factor in production of the pigment.

Unsaturated fatty acids were recognized as important etiologic agents in the production of ceroid and Casselman,[14] in a series of well conceived experiments, demonstrated the *in vitro* preparation of substances resembling ceroid by the auto-oxidation of unsaturated fats, fatty acids, or their esters. Similar reactions were not given by hydrocarbons, saturated fats, or fatty acids. The formation of these ceroid-like substances was inhibited by antioxidants. He concluded that whenever conditions are such that unsaturated fats accumulate in tissues to such an extent that a relative lack of biologic antioxidants results, auto-oxidation of the fats and their conversion to ceroid pigments are favored. In a later paper,[15] in support of this view, Casselman reported that ceroid production was greater on diets with fats of high iodine numbers than with those of low iodine numbers and that the addition of either methylene blue or vitamin E as antioxidants inhibited the formation of ceroid.

Some additional support for Casselman's work is apparent in the reports describing "yellow fat disease" in mink[16] and swine.[17] Both papers reported the formation of ceroid following the use of diets high in the unsaturated fatty acids of fish and both conditions were alleviated by feeding vitamin E.

The mass of data relating ceroid formation to unsaturated fatty acids, particularly those of fish products such as cod-liver oil, led naturally to the question of the occurrence of ceroid in fish. Lovern[18] characterized fish fatty acids as being of the common straight-chain monocarboxylic series with a chain length of 12 to 28 carbon atoms.

With increasing length of chain, the limit of unsaturation becomes progressively greater. In cod-liver oil the fatty acids are approximately 16 per cent saturated and 84 per cent unsaturated. One might conjecture that fish must have a highly efficient method for preventing auto-oxidation of these unsaturated fatty acids or they would be particularly prone to the formation of ceroid. Cumings[19] reported on the effects of vitamin E deficiency in the guppy, *Lebistes reticulatus*. His account did not mention the formation of ceroid-like pigments. Pickford,[20] however, in studies of hypophysectomized killifish, *Fundulus heteroclitus*, described ceroid in both experimental fish and in normal wild fish, suggesting that the occurrence of the pigment may be normal in this species. She also described heavy accumulation of ceroid in certain experimental fish, which was attributed to a possible dietary deficiency or unidentified infection.

Since its completion in 1953, the Salmon Nutrition Laboratory (U. S. Fish and Wildlife Service, Cook, Washington) has been a center for the histopathologic examination of diseased fish from all parts of the United States. During the first year of operation, various fish diseases were examined in several different species. It early became apparent that a yellow granular pigment was present in many hematoxylin and eosin sections. It was of interest, therefore, to determine the relationship of this pigment to ceroid and its incidence in diseased fish.

## Methods and Materials

All tissues were fixed in Bouin's solution, stored in 65 per cent alcohol, and embedded in paraffin. Staining methods were selected from the paper by Endicott and Lillie.[3] Sections were stained for 1 hour in 1:1000 aqueous solutions of the acid dyes orange G, indigo carmine, and phloxine B; and for 1 hour in 1:1000 aqueous solutions of the basic dyes brilliant green, basic fuchsin, and crystal violet. The Ziehl-Neelsen carbolfuchsin stain was used to test for acid fastness. Sudan III and IV were used for examination of sudanophilia. The Lorrain Smith Nile blue sulfate stain for differentiating neutral fats also was utilized. The acid ferricyanide test of Perls was used for iron, and Schmorl's ferric chloride-ferricyanide test for Abnutzungspigmente. Weigert's Gram stain was used; and the Gmelin reaction was observed. The Schiff procedure was employed following performic and periodic oxidation, and without previous oxidation.[4] Tests for fluorescence were not made.

Ceroid-like pigments were observed in fish tissues from a variety of sources, as listed. Only 15 incidents are included in this report although the pigments were seen in sections from many more. Most of

the specimens were received from the disease research station of the U.S. Fish and Wildlife Service Microbiological Laboratory, Kearneysville, West Virginia. Some of these represented specific diseases under experimental conditions, but others were single abnormalities observed in normal populations or moribund individuals from populations affected by unknown diseases. One group was selected for histologic examination from a nutritional experiment. Two were wild fish with external lesions. The others were various diseased fish from both State and Federal hatcheries throughout the United States. In each case in which pigment was observed in a diseased hatchery fish, non-moribund fish of the same species were sectioned to determine if the pigment was present. One fish with focal necrosis in the liver was included, although no pigments were observed in hematoxylin and eosin sections.

Dr. R. D. Lillie graciously submitted a sample of rat liver containing ceroid in phagocytes in areas of incipient scarring, for use as control material in the staining procedures.

Only sufficient information has been included in the case histories to demonstrate that the pigment was present in fish tissues under a wide variety of apparently unrelated conditions.

### BACTERIAL AND VIRUS DISEASES AND TUMORS

#### *Hatchery Fish*

*Case 1.* Adult brook trout, *Salvelinus fontinalis,* with a natural infection of eastern kidney disease that is attributed to an unclassified gram-positive diplobacillus.[21] Pigment granules were observed in phagocytic cells in the spleen. (Fish and Wildlife Service, Berlin, N.H.)

*Case 2.* Fingerling brook trout with an experimental infection of eastern kidney disease. Pigment was scattered throughout the parenchymal cells of the liver, which showed no fatty change. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 3.* Fingerling brook trout inoculated with Seitz filtrate of organs from fish infected with eastern kidney disease. Multiple areas of focal necrosis of unknown etiology were present in the liver, which was not fatty. No pigment was visible in hematoxylin and eosin sections. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 4.* Fingerling rainbow trout, *Salmo gairdnerii,* with recurring bacterial gill disease probably caused by myxobacteria.[22] Pigment was scattered throughout parenchymal cells of the liver, which showed only slight fatty changes. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 5.* Adult brook trout infected with ulcer disease caused by *Hemophilus piscium.*[23] Pigment was scattered throughout the pa-

renchymal cells of the fatty liver and the spleen. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 6.* Fingerling blueback salmon, *Oncorhynchus nerka,* infected with a virus disease causing over 90 per cent mortality.[24] Pigment was present in areas of focal necrosis in the liver, which was not fatty, and scattered throughout the spleen. (Fish and Wildlife Service, Leavenworth, Wash.)

*Case 7.* Adult guppy, *Lebistes reticulatus,* with large malignant hepatoma. Pigment granules were concentrated in foci of necrosis in both the liver and kidneys. The liver was extremely fatty and cirrhotic. (Fish and Wildlife Service, Kearneysville, W.Va.)

### *Wild Fish*

*Case 8.* Adult wild smallmouth bass, *Micropterus dolomieu,* infected with lymphocystis disease, probably of virus origin.[25] Pigment was scattered throughout the liver, which was slightly fatty, in phagocytic and parenchymal cells. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 9.* Adult wild yellow sucker, *Catostomus sp.,* from the Shenandoah River where a high mortality occurred, probably due to a bacterial infection. Multiple areas of focal necrosis were observed in the liver, which was markedly cirrhotic but not fatty. Dense deposits of pigment were present in the necrotic areas. (Fish and Wildlife Service, Kearneysville, W.Va.)

### TOXIC MATERIALS

*Case 10.* Adult rainbow trout with an acute mortality caused by excreted metabolic products from too many fish in a limited water supply. Pigment was observed in small areas of focal necrosis in the liver, which was moderately fatty. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 11.* Rainbow trout fry affected by an unidentified substance in the food or water. No fish pathogens were observed. Pigment was observed in the renal tubular epithelium. (Wyoming State Fish Hatchery, Auburn, Wyo.)

*Case 12.* Fingerling rainbow trout with a chronic disease having high mortality. A massive deposition of sudanophilic material was visible grossly in the liver. However, the liver was not infiltrated by fat. This condition and case 13 may be related to the gossypol content of cottonseed meal which was included in the diet.* (Fish and Wildlife Service, Kearneysville, W.Va.)

* Communication from Dr. S. F. Snieszko, Kearneysville, West Virginia, April, 1955.

*Case 13.* Fingerling rainbow trout with a high mortality attributed to a toxic solvent residue or gossypol in cottonseed meal. Pigment granules were present in phagocytic cells in the spleen. The fish were markedly anemic. (West Virginia State Fish Hatchery, Petersburg, W.Va.)

NUTRITIONAL DEFICIENCIES

*Case 14.* Fingerling rainbow trout on the following diet: fresh beef spleen, 40; fresh beef liver, 10; and dry feeds, 50 parts (fish meal, 45; cottonseed meal, 21; wheat middlings, 20; Standard Brands Type 50B yeast, 10; and sodium chloride, 4 parts). This diet appeared nutritionally adequate, growth was normal, and no symptoms of disease were present. A fine, granular material was observed in the hepatic parenchymal cells, which were moderately infiltrated with fat. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 15.* Fingerling rainbow trout on the same diet as case 14 with the fresh beef spleen replaced by 11.1 parts of fish meal. Subnormal growth and a high rate of mortality indicated that this diet was nutritionally inadequate. No fish pathogens were observed. Pigment was scattered throughout the spleen. (Fish and Wildlife Service, Kearneysville, W.Va.)

*Case 16.* Ceroid in rat liver. (Dr. R. D. Lillie, National Institutes of Health, Bethesda, Md.)

## RESULTS

In 12 of the 14 sections containing ceroid-like pigments, staining reactions were observed nearly identical to those described for ceroid.[8] The exceptions follow. The acid-fast characteristic occasionally was somewhat variable. Most globules of the pigment were brilliantly acid-fast after prolonged decolorization (Figs. 2 and 3), but adjacent particles of pigment often were only a pale pink or colorless. The pigment also had a marked affinity for methylene blue which occasionally masked the acid-fast reaction. In these sections the material was light green in sharp contrast to the blue counterstain. Perls' acid ferrocyanide test for iron was negative in 9 of the 12 tissues. Three of the splenic tissues, cases 6, 13, and 15, gave a strongly positive iron reaction. In these sections there were globules of pigment present which were clearly iron-negative. However, other acid-fast and sudanophilic particles were iron-positive.

Without previous oxidation, the material was Schiff negative or, rarely, very faintly positive. It was strongly periodic acid-Schiff positive without antecedent diastase digestion (Figs. 5 and 7). Following performic acid oxidation, the pigment was Schiff positive but not as

strongly as after periodic acid oxidation. The pigment in paraffin sections resisted prolonged treatment with water, ethyl alcohol, acetone, and xylene.

The staining reactions of the pigment in fish tissue were qualitatively similar to those of ceroid in the rat liver control (Fig. 1). Invariably, however, there was an intrinsic difference in the degree or intensity of staining. The fish pigments generally were stained with less intensity or with less uniformity than the rat ceroid.

The fish ceroid was morphologically similar to previous descriptions of ceroid by various workers. It existed in well defined, single globules and in a finely granular, more diffuse form (Fig. 5). The globular type was more strongly acid-fast. In fact, the globules of pigment were stained more intensely by all stains. The finely granular form was also acid-fast, albeit less so than the globular form. There was little to distinguish between the two forms by the Schiff test following performic or periodic acid oxidation.

Ceroid was found in the liver in areas of focal and diffuse necrosis. The pigment was observed also in liver sections which were not necrotic. In these sections it was found in parenchymal cells and in Kupffer cells. Two of the livers were cirrhotic and contained ceroid. In both cases, however, the ceroid was in focal areas of necrosis and not in the fibrous tissue. One of these livers was the site of a massive hepatoma. The ceroid, however, was not found in the tumor cells. The pigment was scattered throughout the spleen (Fig. 7). The granular type was observed more often in the spleen than in other organs and often appeared to be diffused throughout the reticular stroma. The pigment was observed in the basement epithelium of renal tubules in small, well defined droplets (Fig. 8).

In non-moribund hatchery fish of the same species as the diseased specimens in this report, ceroid was not observed.

Three of the 15 sections did not contain ceroid. In case 2, of eastern kidney disease, a pale yellowish to colorless pigment was observed throughout the parenchymal cells in hematoxylin and eosin preparations. This pigment was not acid-fast or sudanophilic. Case 3 was included in order to examine multiple areas of focal necrosis in the liver. No pigment was observed in these necrotic areas by any of the selected staining techniques. The liver of case 14 was from a fish on a nutritionally adequate diet, as indicated by growth. This section was markedly fatty, as is commonly observed in hatchery fish, and the cytoplasm of the parenchymal cells was markedly granular. These granules, however, gave no staining reaction similar to ceroid.

## Discussion

In mammals, ceroid generally has been seen only in rather well defined experimental conditions. Observations on human necropsy cases form the most notable exception to this statement.[9] In marked contrast, ceroid has been observed in fish in a wide variety of apparently unrelated diseases.

The ceroid observed in fish appears to be identical with that of mammals. It is likely that the iron-positive ceroid, which was observed in sections of spleen only, had combined with other hematogenous pigments. The ceroid of fish is similar morphologically to the ceroid of mammals, occurring in both globular and finely granular forms.

The pigment often is observed in areas of necrosis of the liver. However, necrosis is not necessary for its production in fish since the pigment has been observed without necrosis, and necrosis has been observed without ceroid. The spleen frequently contains ceroid but it has not been observed in conjunction with necrosis of that organ. There does not appear to be a direct relationship between simple fatty changes in the liver and the appearance of ceroid. The pigment was seen in livers densely infiltrated with fat and in livers with no readily observable fatty changes. Similarly, densely infiltrated fatty livers were seen unaccompanied by ceroid deposition. Cirrhosis was seen only rarely in these sections and apparently is not directly implicated in the production of the pigment in fish.

It appears that the species of fish reported here do not normally have ceroid in their tissues. However, nutritional deficiencies, bacterial and virus diseases, toxic products, and other physiologic disturbances readily result in the formation of the pigment. In Pickford's[20] report of ceroid occurring normally in wild killifish, the possibility of a chronic low-grade infection in the wild population cannot be excluded. Although hatchery diets usually have little in common with the food of fish under natural conditions, there is no indication that nutrition was an important cause of the ceroid observed in these fish. In many routine sections of non-moribund hatchery fish on diets producing normal growth, ceroid has never been observed. In the two wild fish in which ceroid was observed, lesions produced by bacterial or virus diseases obviously were present. We have not had an opportunity to examine non-moribund wild fish of these species.

These observations indirectly support Casselman's theory[14,15] that whenever unsaturated fatty acids accumulate in tissues so that a relative lack of biologic antioxidants results, auto-oxidation of the fats and their conversion to ceroid pigments are favored. It appears that fish, with their naturally high content of unsaturated fatty acids, are

particularly susceptible to ceroid formation. The slight differences noted in staining intensity between fish ceroid and rat ceroid are probably related to differences in the composition of the unsaturated fatty acids of the two animals. There is no evidence that a deficiency of antioxidants was involved in the cases reported here. It is probable, however, that in situations of physiologic upset, a breakdown of transportation and enzyme systems could result in local deficiencies of essential nutrients.

This may well explain the occurrence of ceroid in focal degenerative lesions in other animals as well as fish. Little is known for fish of their vitamin E requirements or of those of other biologic antioxidants which they may utilize. This problem is now under investigation at the Salmon Nutrition Laboratory.

In view of the high incidence of ceroid in diseased fish, it is possible that this group of animals is unusually well suited for studies on the etiology of ceroid and possibly for other studies concerning the presence or absence of highly unsaturated fatty acids.

## Summary

A pigment, apparently identical to ceroid, has been observed in fish affected with a wide variety of pathologic states, including nutritional deficiencies, bacterial and virus diseases, and disturbances due to toxic materials.

It appears likely that fish, with their high content of unsaturated fatty acids, are particularly susceptible to the formation of ceroid.

## REFERENCES

1. Lillie, R. D.; Daft, F. S., and Sebrell, W. H., Jr. Cirrhosis of the liver in rats on a deficient diet and the effect of alcohol. *Pub. Health Rep.*, 1941, **56**, 1255–1258.
2. Edwards, J. E., and White, J. Pathologic changes, with special reference to pigmentation and classification of hepatic tumors in rats fed *p*-dimethylaminoazobenzene (butter yellow). *J. Nat. Cancer Inst.*, 1941–42, **2**, 157–183.
3. Endicott, K. M., and Lillie, R. D. Ceroid, the pigment of dietary cirrhosis of rats: its characteristics and its differentiation from hemofuscin. *Am. J. Path.*, 1944, **20**, 149–153.
4. Lillie, R. D. Ethylenic reaction of ceroid with performic acid and Schiff reagent. *Stain Technol.*, 1952, **27**, 37–45.
5. György, P. Experimental hepatic injury. *Am. J. Clin. Path.*, 1944, **14**, 67–88.
6. Endicott, K. M.; Daft, F. S., and Sebrell, W. H. Dietary cirrhosis without ceroid in rats. *Proc. Soc. Exper. Biol. & Med.*, 1944, **57**, 330–331.
7. Endicott, K. M. Similarity of the acid-fast pigment, ceroid, and oxidized unsaturated fat. *Arch. Path.*, 1944, **37**, 49–53.
8. Victor, J., and Pappenheimer, A. M. The influence of choline, cystine, and of alpha-tocopherol upon the occurrence of ceroid pigment in dietary cirrhosis of rats. *J. Exper. Med.*, 1945, **82**, 375–383.

9. Pappenheimer, A. M., and Victor, J. "Ceroid" pigment in human tissues. *Am. J. Path.*, 1946, **22**, 395–413.

10. Mason, K. E.; Dam, H., and Granados, H. Histological changes in adipose tissue of rats fed a vitamin E deficient diet high in cod liver oil. *Anat. Rec.*, 1946, **94**, 265–287.

11. Pappenheimer, A. M., and Schogoleff, C. The testis in vitamin E-deficient guinea-pigs. *Am. J. Path.*, 1944, **20**, 239–245.

12. Edwards, J. E., and Dalton, A. J. Induction of cirrhosis of the liver and of hepatomas in mice with carbon tetrachloride. *J. Nat. Cancer Inst.*, 1942–43, **3**, 19–41.

13. Lee, C. S. Histochemical studies of the ceroid pigment of rats and mice and its relation to necrosis. *J. Nat. Cancer Inst.*, 1950–51, **11**, 339–349.

14. Casselman, W. G. B. The *in vitro* preparation and histochemical properties of substances resembling ceroid. *J. Exper. Med.*, 1951, **94**, 549–562.

15. Casselman, W. G. B. Factors influencing the formation of ceroid in the livers of choline-deficient rats. I. Dietary fats. *Biochim. et biophys. acta*, 1953, **11**, 445–447.

16. Gorham, J. R.; Baker, G. A., and Boe, N. Observations on the etiology of yellow fat disease in mink: preliminary report. *Vet. Med.*, 1951, **46**, 100–102.

17. Davis, C. L., and Gorham, J. R. The pathology of experimental and natural cases of "yellow fat" disease in swine. *Am. J. Vet. Research*, 1954, **15**, 55–59.

18. Lovern, J. A. The Chemistry and Metabolism of Fats in Fish. In: The Biochemistry of Fish, Williams, R. T. (ed.). Biochemical Society Symposia No. 6, Cambridge University Press, Cambridge, 1951, pp. 49–62.

19. Cumings, H. W. The effects of vitamin E deficiency in the guppy, *Lebistes reticulatus*. *Anat. Rec.*, 1942, **84**, 499–500.

20. Pickford, G. W. Fish endocrinology. A study of the hypophysectomized male killifish, *Fundulus heteroclitus* (Linn). *Bull. Bingham Oceanographic Coll.*, 1953, **14**, no. 2, 5–41.

21. Earp, B. J.; Ellis, C. H., and Ordal, E. J. Kidney disease in young salmon. Washington State Department of Fisheries, 1953, Special Report Series 1, 74 pp.

22. Rucker, R. R.; Johnson, H. E., and Kaydas, G. An interim report on gill disease. *Prog. Fish-Cult.*, 1952, **14**, 10–14.

23. Snieszko, S. F. Ulcer disease in brook trout (*Salvelinus fontinalis*): Its economic importance, diagnosis, treatment, and prevention. *Prog. Fish-Cult.*, 1952, **14**, 43–49.

24. Watson, S. W.; Guenther, R. W., and Rucker, R. R. Virus Disease of Sockeye Salmon: Interim Report. Special Scientific Report—Fisheries, 1954, No. 138, 36 pp. USDI, Washington, D.C.

25. Davis, H. S. Culture and Diseases of Game Fishes. University of California Press, Berkeley, 1953, pp. 292–293.

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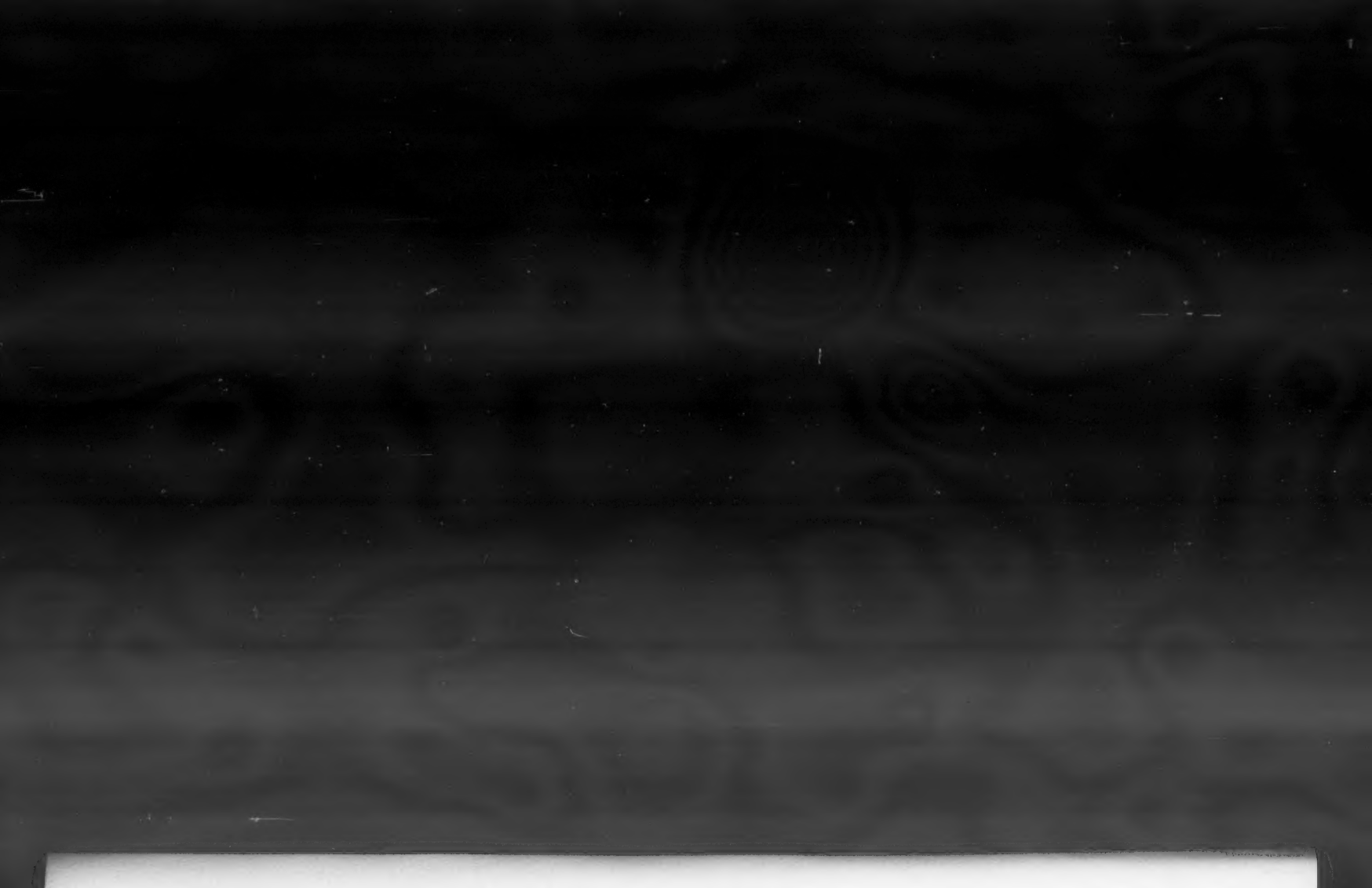
## LEGENDS FOR FIGURES

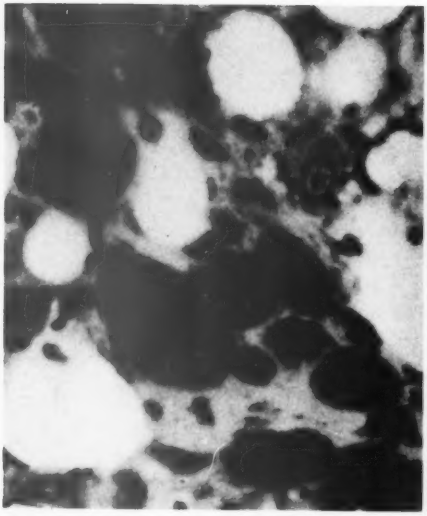
FIG. 1. Case 16. Ceroid in rat liver control. Acid-fast stain. × 500.

FIG. 2. Case 12. Ceroid in fish liver. Acid-fast stain. × 250.

FIG. 3. Case 12. Ceroid in fish liver. Acid-fast stain. × 500.

FIG. 4. Case 12. Ceroid in fish liver. Sudan IV stain. × 250.





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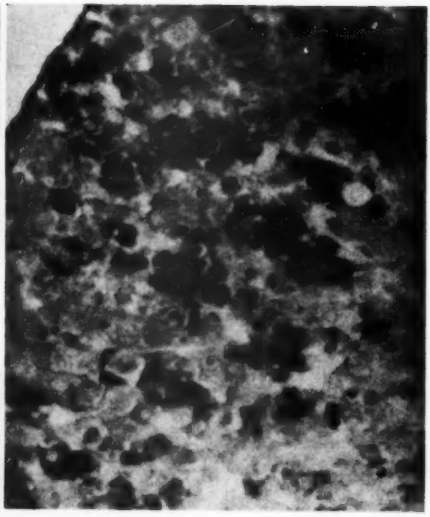

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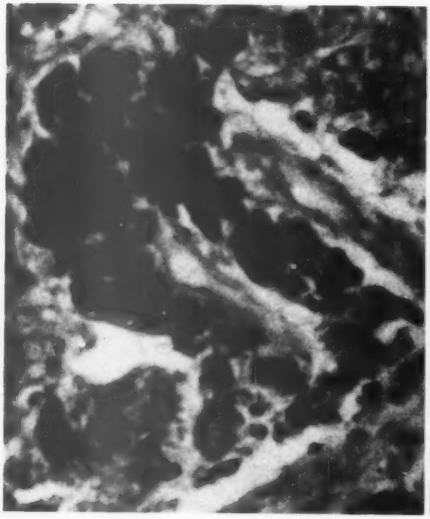

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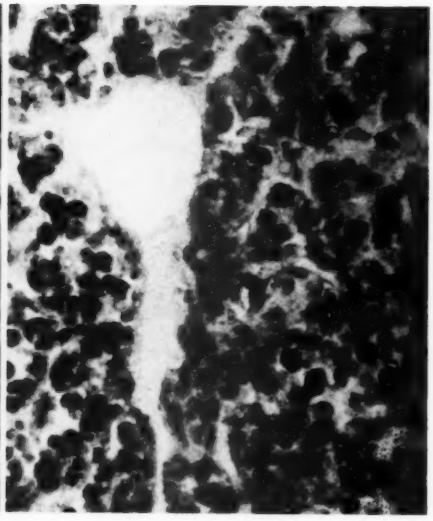

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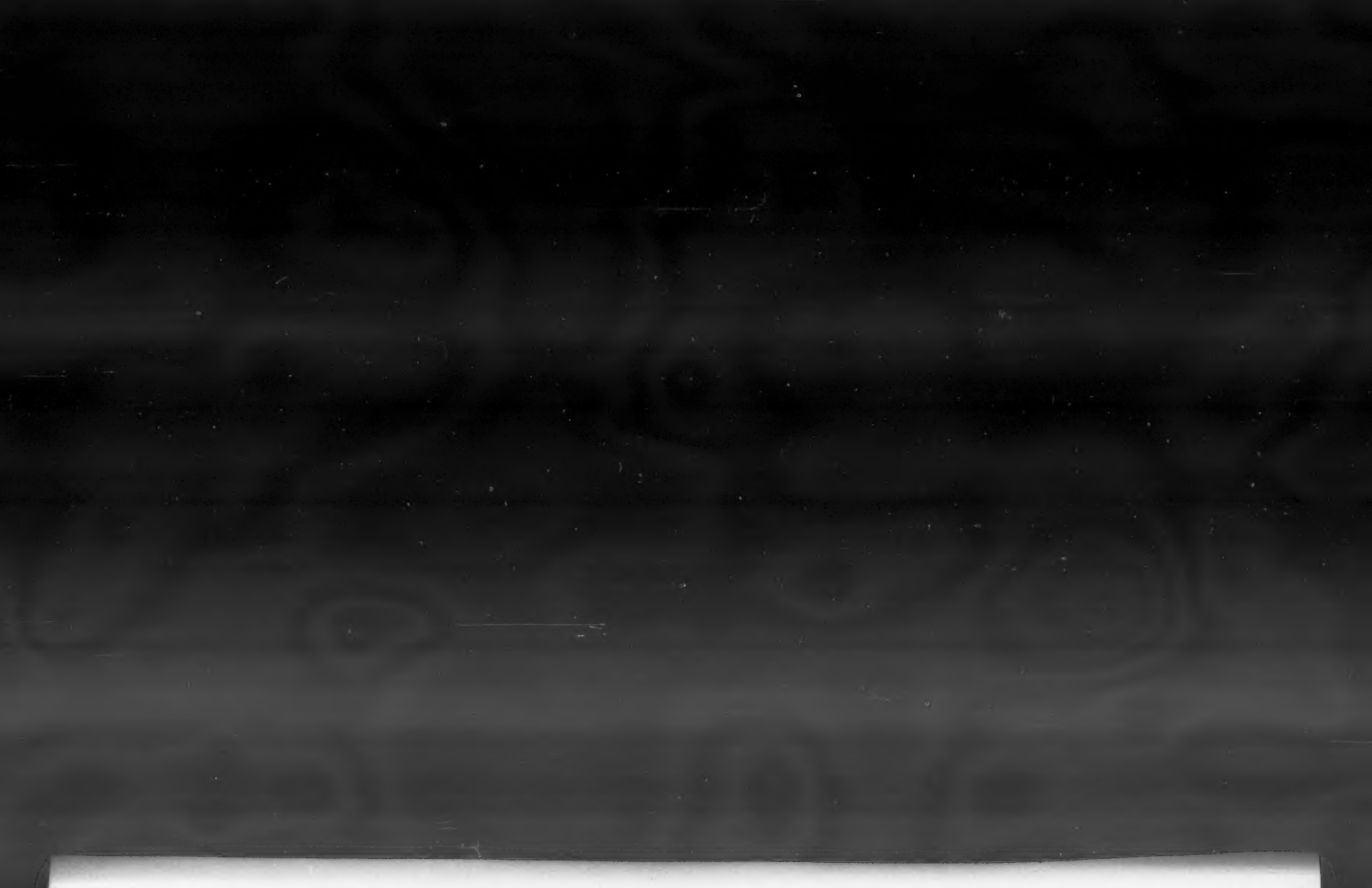
FIG. 5. Case 5. Ceroid in fish liver, showing a finely granular form. Schiff's stain after periodic oxidation. × 2,000.

FIG. 6. Case 7. Ceroid in fatty fish liver. Basic fuchsin stain. × 1,000.

FIG. 7. Case 6. Ceroid in fish spleen. Schiff's stain after periodic oxidation. × 2,000.

FIG. 8. Case 11. Ceroid in fish renal tubules. Sudan IV stain. × 500.



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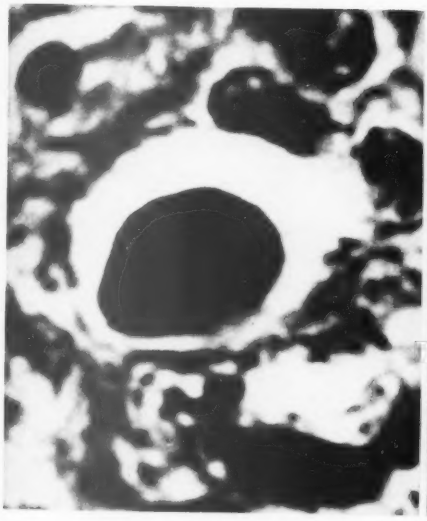

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# FIFTY-THIRD ANNUAL MEETING
# OF THE
# AMERICAN ASSOCIATION OF PATHOLOGISTS
# AND BACTERIOLOGISTS

CINCINNATI, OHIO

APRIL 26TH, 27TH, AND 28TH, 1956

# THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Fifty-third Annual Meeting
HOTEL NETHERLAND PLAZA
Cincinnati, Ohio
April 26th, 27th, and 28th, 1956

PRESIDENT SCHULTZ IN THE CHAIR

## BUSINESS MEETING

April 26, 1956

The following nominations for elective officers were submitted by the Council:

| | |
|---|---|
| *President* | DR. GRANVILLE A. BENNETT |
| *Vice-President* | DR. SIDNEY FARBER |
| *Secretary* | DR. EDWARD A. GALL |
| *Treasurer* | BRIG. GEN. ELBERT DECOURSEY |
| *Incoming Member of Council* | DR. JOHN G. KIDD |

Additional nominations were called for. None having been offered, it was moved and seconded from the floor that the nominations be closed. (At a supplementary business session it was further moved, seconded, and directed that the Secretary be instructed to cast a unanimous ballot for the entire slate.)

At the direction of the President, the Secretary reported the following actions of the Council:

### Election of New Members

| | |
|---|---|
| Abell, Murray R. | Crowson, Charles N. |
| Anderson, William | Feenstra, Ernest S. |
| Ashley, Charles A. | Godman, Gabriel C. |
| Bencosme, Sergio A. | Gueft, Boris |
| Benirschke, Kurt | Hadlow, William J. |
| Blundell, G. P. | Hales, Milton R. |
| Boley, J. O. | Hanan, Robert |
| de Brux, Jean A. | Harris, Charles |
| Christopherson, William A. | Haukohl, Robert S. |
| Clark, W. H. | Hendrix, Robert C. |
| Cooper, Norman S. | Jonas, David B. |

Kalfayan, Bernard
Maniglia, Rosario
Mann, Frank D.
Martens, Vernon E.
McCluskey, Robert T.
McGill, Henry C., Jr.
Miale, John B.
Milstone, J. Haskell
Morgan, W. S.
Nigrelli, Ross F.
O'Neal, Robert M.
Opler, Stanley R.
Penick, G. D.
Plachta, Aaron
Pollak, Ann
Rabson, Alan S.
Race, George J.
Russfield, Agnes B.
Shubik, Philippe
Smith, Joseph F.
Song, Yo Seup
Sprinz, Helmuth
Wagner, Bernard M.
Walford, Roy L., Jr.
Zeman, Wolfgang

The Secretary announced the resignation of the following members:

Barron, Moses
Clowes, George H. A.
Jacobsen, Victor
Lamb, Frederick H.
Loeb, Emily Nichols
Morales-Otero, Pablo

With deep regret the deaths of the following members were recorded:

Jacobson, Conrad
Lowenhaupt, Elizabeth
Norris, Edgar H.
Paige, Beryl H.
Pratt, Joseph H.
Rinehart, James F.
Rosenthal, Nathan
Thorp, Frank, Jr.
Weiss, Emil
Werne, Jacob

The Secretary announced the following actions by the Council:

The re-election of Dr. Ernest W. Goodpasture to the Editorial Board of *The American Journal of Pathology* for a period of six years, beginning January 1, 1957.

The re-election of Miss Dorothy E. Seiferlein as Editorial Assistant of *The American Journal of Pathology* for the ensuing year.

The election of Dr. Benjamin H. Landing as Assistant Secretary of the Association for the ensuing year.

The re-election of Dr. Elson B. Helwig as Assistant Treasurer of the Association for the ensuing year.

The Secretary announced that the next annual meeting of the Association will be held in Washington, D.C., on April 11, 12, and 13, 1957. The topic for the symposium is "Diseases Caused by Environmental Factors (Dusts, Gases, and Other Physical and Chemical Agents)."

The Secretary further announced that the annual meeting in 1958

will be held in Cleveland, Ohio, on April 24, 25, and 26. The topic for the symposium in 1958 will be "Natural and Acquired Factors in Resistance to Disease."

The President then asked if there was any business from the floor. None was presented, and the business meeting adjourned.

Edward A. Gall, *Secretary*

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## REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a letter of certification from Ralph Cole, Certified Public Accountant, of Washington, D.C. In condensed form, the Treasurer's report follows:

### General Checking Account

*Receipts*

| | | |
|---|---|---|
| Balance on hand, January 1, 1955 | | $ 6,277.21 |
| Membership dues | $ 8,909.31 | |
| Interest on bonds and savings accounts | 905.10 | |
| Transfer from investment account | 10,000.00 | |
| | | 19,814.41 |
| Total receipts | | $26,091.62 |

*Disbursements*

| | | | |
|---|---|---|---|
| American Journal of Pathology | | $ 7,128.00 | |
| Secretary's office, clerical | $ 415.75 | | |
| Printing, travel, supplies | 956.71 | | |
| | | 1,372.46 | |
| Treasurer's office, clerical | 150.00 | | |
| Bonding and auditing | 153.00 | | |
| Printing, supplies, miscellaneous | 173.60 | | |
| | | 476.60 | |
| Miscellaneous, expenses of referee | 214.00 | | |
| American Type Culture Comm. | 200.00 | | |
| Investment in Bldg. & Loan Shares | 10,000.00 | | |
| | | 10,414.00 | |
| Total disbursements | | | $19,391.06 |
| Balance on hand, December 31, 1955 | | | $ 6,700.56 |

Investment Inventory

| | | |
|---|---|---|
| U.S. Treasury Bonds, series G, due January, 1957 | | $20,000.00 |
| Savings accounts | | |
| Riggs National Bank, Washington | $ 1,254.47 | |
| National Bank of Washington | 3,591.86 | |
| Olympic Savings & Loan Assoc., Berwyn, Ill. | 5,000.00 | |
| Home Savings & Loan Assoc., Los Angeles | 5,000.00 | |
| | | 14,846.33 |
| Earned but unrealized interest on U.S. bonds | | 250.00 |
| Total of investment inventory | | $35,096.33 |

Elbert DeCoursey, *Treasurer*

# SCIENTIFIC PROCEEDINGS

## ABSTRACTS

PULMONARY ALVEOLAR MICROLITHIASIS IN MITRAL STENOSIS. Raymond Yesner* and Alan B. Skorneck, Veterans Administration Hospital, West Haven, Conn.

The literature on pulmonary alveolar microlithiasis, on analysis, is found to refer to two varieties of calculi. In one form, the stones are oval, laminated, and strongly resemble corpora amylacea. They occur in large numbers in a variety of pulmonary diseases, and although bone formation occurs, it is not common. The second variety occurs almost exclusively in mitral stenosis due to rheumatic heart disease. The calculi are uneven, bossed structures, and ossification is common. Alveolar microlithiasis may be readily distinguished from calcified granulomas and septal calcification of the aged. Theories have been proposed that these calculi are derived from calcified Masson bodies, from small thrombosed vessels extruded into air spaces, and from alveolar exudate. Two cases of the mitral stenosis variety of pulmonary alveolar microlithiasis are described, with roentgenologic evolution, and studies of the location and structure of the stones, indicating derivation from alveolar exudate.

IDIOPATHIC PULMONARY HEMOSIDEROSIS. Simon Russi* and Charlie F. Wingo, Veterans Administration Hospital, Richmond, Va.

A 37-year-old white male was hospitalized on numerous occasions over a period of about 7 years because of recurrent attacks of fever, cough, and pulmonary hemorrhage, associated with weakness, anemia and often cyanosis. Post-mortem examination revealed marked deposition of hemosiderin throughout the pulmonary parenchyma and regional lymph nodes. There was, also, much morphologic alteration of the pulmonary vessels with degeneration of the elastic fibers. There were giant cells of the foreign body type around degenerated elastic fibers. There was, also, considerable impregnation of the elastica with iron pigment. Acute and chronic pneumonitis accompanied the deposits of iron pigment. The condition appeared morphologically identical with the idiopathic hemosiderosis described almost exclusively in children. Only one case of 30 previously reported cases occurred in an adult.

ENDOGENOUS "PNEUMOCONIOSIS" ASSOCIATED WITH ALTERED ELASTIC TISSUE. Roy L. Walford and Leo Kaplan, University of California School of Medicine and Mount Sinai Hospital, Los Angeles, Calif.

The elastic tissue throughout the lungs may be altered and appear to act as a foreign body under certain conditions, provoking a severe interstitial and vascular pulmonary sclerosis. This process is characterized histologically by basophilia, increase in number of fibers and thickening and fragmentation of pulmonary elastic tissue, encrustation of elastic tissue with iron compounds but not with calcium, and phagocytosis of elastic tissue fragments by foreign body giant cells. This type of elastica alteration bears some similarity to Gamna-Gandy body formation, the pulmonary hemosiderosis associated with mitral stenosis, Ceelen's idiopathic pulmonary hemosiderosis of children, and possibly to pseudoxanthoma elasticum.

Twelve cases of endogenous "pneumoconiosis" apparently caused by altered elastic tissue are presented, ranging in age from 24 to 73 years. Four of these appeared to be primary in the lungs, as they were unassociated with cardiac pathology other than a secondary cor pulmonale. Eight were associated with left-sided heart disease, including mitral stenosis, luetic valvulitis, hypertensive heart disease, and advanced coronary arteriosclerosis. It seems probable that damaged or degenerating

* Asterisks indicate members of The American Association of Pathologists and Bacteriologists. All others appear on the program "by invitation."

pulmonary elastic tissue has an affinity for hemosiderin (hence the basophilia) so that when a high local concentration of hemosiderin is available, the pathologic elastica changes are more easily promoted or delineated. However, the rôle of hemosiderin in provoking pulmonary elastica disease of this type was at most contributory, as a number of the cases with severe elastica changes showed only mild pulmonary hemosiderosis. Specific causes of the elastica alteration have not yet been discovered; but once progression to the stage of "pneumoconiosis" has occurred, the process appears to be self-sustaining. It may end in death from pulmonary insufficiency.

ANATOMICAL AND FUNCTIONAL STUDIES OF LUNGS DEPRIVED OF PULMONARY ARTERIES AND VEINS, WITH AN APPLICATION IN THE THERAPY OF TRANSPOSITION OF THE GREAT VESSELS. Romeo A. Vidone and Averill A. Liebow,* Yale University School of Medicine, New Haven, Conn.

The present experiments demonstrate that, in the dog protected with penicillin and streptomycin, the left lung will survive (without necrosis) the interruption of all pulmonary arterial inflow and all pulmonary drainage. The lung soon acquires a collateral blood supply from the aorta and a collateral venous drainage into the azygos and superior vena caval systems. Of particular interest is the development of a large retrocardiac arterial bridge between bronchial arteries and the left circumflex coronary artery. The inflow of blood across this bridge into the coronary arteries has been found as high as 2.6 per cent of the total coronary flow. Respiratory function is quickly regained. Measurements of pulmonary blood flow, by appropriate methods of bronchospirometry and blood gas analysis, demonstrate flows increasing progressively in 16 months' time, to a range approximating 1 l. per minute.

Since the lung of the operated side receives its blood from the aorta and delivers it to the right atrium, an application to the therapy of congenital transposition of the great vessels suggests itself. In this condition, such a lung would function as an automatic oxygenator within the transposed systemic circuit.

EXFOLIATIVE PNEUMONIA. Bernhard Steinberg,* Toledo Hospital Institute for Medical Research, Toledo, Ohio.

A morphologic study of two patients with pulmonary disorders is presented. The lung lesions are unusual and consist essentially of an exfoliation of bronchial and alveolar mucosal lining cells. The attendant inflammation is limited and restricted. Other abnormal changes of the lungs complete a morphologic picture which suggests either a poorly differentiated or a hitherto undescribed pulmonary lesion. Comparative analyses are presented of the changes described in atypical (virus) pneumonia; meningopneumotropic, psittacotic, and psittacosis-like pneumonias; and in certain rickettsial disorders such as scrub typhus and tularemia. On the basis of morphologic changes, exfoliative pneumonia (the term suggested for this disorder) and other atypical pneumonias are grouped and classified.

MONONUCLEAR PNEUMONIA OF INFANTS. Peter Gruenwald,* Margaret Hague Maternity Hospital, Jersey City, N.J.

A diffuse, mononuclear pneumonia is the characteristic necropsy finding in infants who died either suddenly without known disease, or after a fulminating illness characterized by increasing respiratory distress. The cause is unknown. There is no evidence for a physical, chemical, or bacterial causative agent, and the possibility of a virus has been suggested. The problem is complicated by finding a similar pneumonia in other infants. It is therefore not known whether necropsy findings of mononuclear pneumonia are indications of a highly significant change.

In order to furnish a basis for further work and to bring the problem into focus, 678 unselected necropsies on infants older than 1 week were investigated as to the occurrence of mononuclear pneumonia, other pathologic findings, and certain points

in the clinical history. The incidence of various associated pathologic lesions as well as the frequency of sudden death, fulminating respiratory disease, prodromal "colds," and other pertinent clinical observations was determined. The results suggest that the disease producing mononuclear pneumonia is not always the primary cause of death. Infants dying with malformations, or following operations, frequently show this condition; it is usually present in infants with fatal purulent meningitis. Necropsies on older children and adults show mononuclear pneumonia with decreasing frequency. It is suggested that many infants with the same basic disease may manifest only non-characteristic, mild signs such as a "cold," and recover.

All stages of transition from typical mononuclear pneumonia to lobar pneumonia have been seen; the latter occurs in instances of longer survival, presumably by superimposed bacterial infection. This recalls the opinion of several experts that lobar pneumonia is caused by a combination of viral and bacterial infection.

In view of the predominance of mononuclear pneumonia in infants, virologic and epidemiologic examination of fetal as well as possible non-fetal cases and their contacts is imperative. A comprehensive investigation of this condition, which figures so prominently in infant mortality, should be of scientific interest and medical importance.

PULMONARY HYALINE MEMBRANE DISEASE: A MORPHOLOGIC STUDY UTILIZING THE ELECTRON MICROSCOPE. H. B. Neustein and V. L. van Breemen, University of Colorado Medical Center, Denver, Colo.

Pulmonary hyaline membranes were the only pathologic finding in 40 per cent of deaths of premature infants according to Potter. Analysis of similar material at the Colorado General Hospital showed an incidence of pulmonary hyaline membranes of approximately 25 per cent and no other significant pathologic findings in necropsies of premature infants. Under light microscopy with hematoxylin and eosin stain, the pulmonary hyaline membrane consists of a homogeneous eosinophilic membrane lining alveolar ducts and alveoli. In the human being, pulmonary atelectasis and hyperemia are usually concomitant findings. The etiology of these membranes is poorly understood. In order to clarify the nature of these membranes a morphologic study of normal and pathologic lung tissue from guinea-pigs and premature infants was made utilizing the electron microscope.

Lung tissue was obtained from premature infants dying in this hospital. Pulmonary hyaline membranes, produced in guinea-pigs by the method described by Bruns and Shields, were used for comparison. Unstained osmic acid fixed, methacrylate-embedded, lung sections were examined under the electron microscope. Similarly fixed and embedded lung sections stained by hematoxylin and eosin, Gomori's trichrome, and periodic-acid Schiff's methods were examined under the light microscope for comparison. Low's observations of normal lung in the guinea-pig and human being were corroborated. Epithelial cells lined alveoli and rested on a definite basement membrane. The attenuated cytoplasm of these epithelial cells could be followed in the alveolus, but a definite statement cannot be made as to whether or not the lining epithelium is *continuous*.

Our studies with the electron microscope indicate that the pulmonary hyaline membranes of human infants and of guinea-pigs are essentially the same. The hyaline membrane consists of a mass of fibrils, representing fibrin, and cell débris overlying the epithelium of alveolar ducts and some alveoli. The alveolar ducts and some alveoli contained fine granular material (probably plasma protein) and fibrils extended from this material into the hyaline membrane.

FIBRINOID AND HYALIN: A DIFFERENTIATION ACCORDING TO CHARACTERISTICS. P. O'B. Montgomery* and E. E. Muirhead,* the University of Texas Southwestern Medical School, Dallas, Texas.

A group of histochemical procedures have been applied to "hyaline" and "fibri-

noid" lesions derived from multiple sources. Two types of hyalin (vascular and connective tissue) and two types of fibrinoid (vascular and connective tissue) have been characterized. The vascular hyalin was derived from the arteriolar hyalin in the kidneys of 6 patients with benign arteriolar nephrosclerosis and Kimmelstiel-Wilson disease. The connective tissue hyalin was obtained from an old scar, a meningioma, 2 keloids, 3 cases of scleroderma, and one uterine fibroid. The vascular "fibrinoid" was obtained from 11 cases of malignant hypertension. The connective tissue "fibrinoid" was obtained from the centers of two subcutaneous nodules of rheumatoid arthritis and from the base of an acute gastric ulcer. The two types of hyalin and "fibrinoid" were compared to fibrin as deposited on the pericardium in 4 cases of uremic pericarditis.

The results indicate three separate histochemical patterns: 1. Similar characteristics for vascular hyalin and vascular "fibrinoid." 2. A separate pattern of characteristics for fibrin and connective tissue fibrinoid. 3. A third pattern for connective tissue hyalin.

The vascular lesions contain large amounts of lipid material as indicated by positive staining with oil red O, Nile blue sulfate, and Sudan black B and by a positive Schultz reaction; while the connective tissue lesions and fibrin are essentially negative with these procedures. The vascular lesions contain, in addition, free carbonyl groups, Congo red positive material, protein bound SH groups, and potassium. The fibrin and connective tissue lesions contain none or greatly reduced amounts of these substances. The fibrin and connective tissue fibrinoid contain tyrosine, indole, and calcium. These substances are essentially absent from the vascular lesions and connective tissue hyalin.

It is concluded that there are two types of hyalin and two type of "fibrinoid." The vascular fibrinoid and hyalin have been considered to be derived mainly from smooth muscle. Connective tissue "fibrinoid" appears to consist mainly of fibrin, a confirmation of the observations of Gitlin and Craig.

THE NATURE AND ORIGIN OF FIBRINOID. Henry Z. Movat and Robert H. More,* Queen's University, Kingston, Ont.

Much controversy exists concerning the pathogenesis of fibrinoid. The various theories propounded derive it from collagen, ground substance, fibrin, or nucleoprotein. The following observations on the nature of fibrinoid were made on human and experimental fibrinoid.

Experimentally, fibrinoid was produced by a number of methods all causing connective tissue injury: mechanical injury to the skin following incision, chemical injury following injection of trypsin, and radiation injury after insertion of radon seeds. Allergic injury of both skin and joint was produced by eliciting the Arthus phenomenon. Systemic lesions of hypersensitivity with fibrinoid were produced by injecting intravenously massive doses of protein. Fibrinoid in a number of lesions from the "diffuse collagen diseases" also was examined.

The experimental lesions were studied at serial time intervals. In all types, the earliest change was an acute inflammation in which varying amounts of fibrin could be demonstrated. At later stages, fibrin had conglomerated and inspissated, thus having all the characteristics of fibrinoid. It was acidophilic (eosinophilic), homogeneous, and refractile, and gave staining reactions similar to fibrin. It stained red with the trichrome, dark blue with phosphotungstic acid hematoxylin, and red-purple with PAS. With the aid of histochemical methods, the amino acids tyrosine, tryptophane, cysteine, and cystine could be demonstrated in both reticular fibrin and homogeneous fibrinoid. Incubation of alcohol-fixed sections with trypsin resulted in digestion of both fibrin and fibrinoid while collagen persisted. Acid mucopolysaccharides (AMP) were decreased in the earliest exudative lesions and increased as regeneration and repair commenced, and young connective tissue was laid down. Fibrinoid was embedded in this AMP-rich material. Following hyaluronidase incu-

bation, the staining of AMP was inhibited or diminished, but the fibrinoid remained unchanged.

In the collagen diseases, all stages of fibrinoid formation could be traced from delicate threads of fibrin to bands, clumps, and large masses which showed all the morphologic and tinctorial characteristics of fibrinoid described. Histochemically, fibrin and fibrinoid gave all the reactions found in the experimental fibrinoid. AMP was a prominent feature only in the advanced lesion. A hematoxylinophilia was frequently associated with fibrinoid in disseminated lupus, but it was found at times also in rheumatoid arthritis and periarteritis nodosa. It always gave a positive Feulgen reaction. Possibly the most significant finding was the fibrinolysin digestion of fibrinoid of alcohol-fixed subcutaneous nodules of rheumatoid arthritis. Trypsin had a similar effect.

Acid mucopolysaccharides can be demonstrated only in advanced lesions having fibrinoid. The reason for this is that they are associated with repair, i.e., formation of young connective tissue. The demonstration of increased amounts of AMP in association with fibrinoid has led some investigators to conclude that fibrinoid is derived from a precipitation of AMP. However, in the early exudative lesions, AMP is diminished or, at least, not demonstrable by histochemical methods. From these studies there is ample morphologic evidence that fibrinoid derives from conglomeration and inspissation of fibrin. Histochemical evidence supports this view. The amino acids which can be demonstrated histochemically in fibrinoid are present in fibrin, but are absent or in low concentration in connective tissue. In alcohol-fixed tissue, fibrinoid is digested by trypsin and fibrinolysin.

It was concluded from these studies that fibrinoid derives from fibrin which is exuded during the course of a fibrinous or serofibrinous inflammation. There is little evidence that fibrinoid derives from collagen or connective tissue ground substance. Degenerating elements of connective tissue, i.e., collagen and ground substance, and also nuclear material, can be separated readily from fibrinoid.

STUDIES ON THE IMMUNOHISTOCHEMICAL COMPOSITION OF INFLAMMATORY AND DEGENERATIVE LESIONS.† Jacinto J. Vazquez and Frank J. Dixon,* University of Pittsburgh School of Medicine, Pittsburgh, Pa.

The "fluorescent antibody" technique of A. Coons and associates, with slight modifications, was applied to the study of the gamma globulin and albumin content of tissue lesions in secondary human amyloidosis; experimental rabbit amyloidosis; and some diseases in the so-called "collagen group." Emphasis was placed on evaluating the specificity of the technique, using both a variety of normal and pathologic tissues and fluorescent antisera. Immunologic characterization of fluorescent antibody was accomplished by the precipitin reaction in fluid and gel media.

Amyloid deposits in 2 cases of secondary human amyloidosis as well as in experimentally produced amyloidosis in rabbits, after injections of casein or RNA (ribonucleic acid), stained strongly for the corresponding gamma globulin and weakly for albumin. The kidney in a case of lupus erythematosus showed considerable concentration of gamma globulin and relatively little albumin in areas of fibrinoid change in arterioles and glomeruli. Subcutaneous nodules of rheumatoid arthritis showed considerable gamma globulin and less albumin, particularly in areas of fibrinoid change. In 2 children dying from rheumatic heart disease, the altered heart valves as well as connective tissue in the myocardium showed concentration of gamma globulin and little albumin. In comparable normal tissues there was more staining for albumin than for gamma globulin. In dense scar tissues, preliminary studies showed little evidence of specific staining of either albumin or gamma globulin. Considerable amounts of albumin with less gamma globulin were present in the exudate of a case of acute appendicitis. More gamma globulin than albumin,

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however, was found in the chronically inflamed tissue of the wall of a tuberculous pulmonary cavity.

The significance of the observed concentrations of gamma globulin in the situations described cannot as yet be determined. The possibility that they may represent localized antibody is suggestive and is under investigation.

TESTS FOR RÔLE OF HEPARIN AND PROTEIN-BOUND POLYSACCHARIDE CORRELATED WITH C-REACTIVE PROTEIN AND WELTMANN SEROCOAGULATION BAND. Tom R. Hamilton,* Antoni M. Diehl, Robert W. Weber, Irvin Snyder, Charles Orthwein, Robert Olson, Francis O'Kane, and John S. May, University of Kansas School of Medicine, Kansas City, Kans.

The blood of children and adults has been studied to elucidate the evaluation of activity of reactive states in various stages. Experience in the year 1955 has centered upon the results of 4,662 tests upon 1,515 specimens. Attention has been focused especially upon so-called collagen diseases, particularly various stages of the rheumatic state, endocarditis, rheumatoid arthritis, and lupus erythematosus. The study has included investigation of other conditions which may yield fundamental information or enter into differential diagnosis.

The correlation may be visualized by tabulating incidences (percentage) of positive tests for C-reactive protein and cold heparin precipitate and increased values (expressing abnormal levels) of protein-bound polysaccharide (Anthrone method) and heparin tolerance. These figures indicate striking differences in values obtained on bloods with left shift in Weltmann reaction, viz., shortened serocoagulation band (tubes 1, 2, or 3) in contrast to normal band (tubes 6 and 7).

| | Marked left shift | | Normal | Right shift |
|---|---|---|---|---|
| Weltmann tube numbers | 1, 2, and 3 | 4 and 5 | 6 and 7 | 8, 9, and 10 |
| C-reactive protein | 66% | 36% | 16% | 24% |
| Cold heparin precipitate | 61 | 39 | 21 | 36 |
| Heparin tolerance | 60 | 51 | 38 | 40 |
| Protein-bound polysaccharide | 89 | 53 | 32 | 48 |

These differences are statistically significant employing the chi-square test for analysis of the data.

Correlation between degrees of positivity is found in comparing C-reactive protein (C-RP) and cold heparin precipitate (CHP), i.e., at the 4 plus level of CHP, 75 per cent of the same bloods yielded positive C-RP of which the majority were greater than 2 plus; at the level of 1 plus CHP, only 38 per cent were associated with positive C-RP and one fourth were greater than 2 plus C-RP. Between protein-bound polysaccharide and C-RP, correlation is indicated by median level of 193 mg. per cent (P-BP) in bloods with positive C-RP in contrast with normal 149 mg. per cent associated with negative C-RP. Similarly, cold heparin precipitate is associated with a median protein-bound polysaccharide level 20 mg. per cent higher than normal value of 153 mg. per cent, which is the median value yielded by bloods with negative CHP.

Further studies are being performed with emphasis upon mechanism and significance of tests as well as another phase especially focused upon correlation with clinical activity of the disease processes.

ON THE COMPOSITION OF THE HEMATOXYLIN BODIES OF SYSTEMIC LUPUS ERYTHEMATOSUS. Gabriel C. Godman, Arline D. Deitch, and Paul Klemperer,* Columbia University, College of Physicians and Surgeons, and the Mount Sinai Hospital, New York, N.Y.

Typical hematoxylin staining masses in the kidney, blood vessels, and lymph nodes from fatal cases of systemic lupus erythematosus, as well as lupus bodies prepared from blood by different techniques, were studied cytochemically employing succes-

sive staining schedules on the same smears or sections. The lupus bodies are derived from nuclei and have been shown to contain desoxyribosenucleic acid (DNA). Microspectrophotometric measurements confirmed that the ratio of the amount of DNA in them, determined by Feulgen test, to that found by methyl green staining, was higher than that of unaffected nuclei, owing to depression of methyl green binding by DNA in the lupus bodies. Acetylation of the basic (amino) groups of the proteins resulted in approximately two-fold increases of methyl green uptake by DNA of the same bodies, and consequent revision of their Fuelgen:methyl green ratios to values similar to those of control nuclei. These determinations indicate that the decreased methyl green binding and consequently increased Feulgen: methyl green ratios of lupus bodies result from the presence of proteins whose basic groups compete with the dye for the binding sites of DNA, and so inhibit staining. Since, after acetylation, Feulgen:methyl green ratios of lupus bodies are about 1.0, as in normal control nuclei, protein interference alone and not depolymerization of DNA accounts for lowered methyl green staining. Photometric comparison of the mean Feulgen-stainable DNA content per lupus body with that of control nuclei shows that the former contain less DNA; however, this DNA is not appreciably changed in physical state.

Application of the alkaline fast green technique for histone (Alfert and Geschwind, 1953) after the TCA modification of the Feulgen reaction (Bloch and Godman, 1955) did not reveal the presence of any histone in the lupus bodies; in normal and pyknotic nuclei, however, this protein was found wherever DNA was present, and in the same sites. In the formation of the lupus body from a nucleus, the histone is believed to be either degraded and lost, or masked by some abnormal protein. The nucleoprotein constitution of the hematoxylin body differs profoundly from that of normal nuclei in the apparent absence of histone, some loss of DNA, and the presence, in seemingly increased amount, of unusual protein material. It is probable that in the nuclear alteration of lupus the initial changes occur in the proteins of the nucleus rather than the nucleic acids.

THE ASSOCIATION OF GRANULATION TISSUE GROUND SUBSTANCE WITH FIBROBLASTIC ACTIVITY. H. E. Taylor* and A. M. Saunders, University of British Columbia, Vancouver, B.C.

The ground substance of granulation tissue presumably consists of acid mucopolysaccharide complexes. It is thought to be produced by either fibroblasts or tissue mast cells. This report is based on the results of a morphologic study of granulation tissue formed about gel foam pledgets, implanted subcutaneously into Wistar strain rats. Groups of these animals were sacrificed 1, 2, 3, 7, and 14 days after the implant and the ground substance, cellular reaction, and fibril formation occurring at the periphery of the implant were studied in serial sections. The battery of staining methods included a modified Hale colloidal iron method for acid mucopolysaccharides, toluidin blue, periodic acid Schiff's reagent, silver impregnation for reticulin, and Masson's trichrome stain. It was concluded that the ground substance mucopolysaccharides were formed through the influence of fibroblasts during a relatively short period of their functional activity, while the fibroblasts were still immature and morphologically resembled reticulo-endothelial cells. There was no evidence that tissue mast cells were directly concerned in this process. The colloidal iron method proved to be much superior to toluidin blue in demonstrating the "metachromatic" ground substance, while the latter did not appear to react with the PAS method.

A SIMPLE, RAPID, MULTIPURPOSE STAIN. William Antopol,* Beth Israel Hospital, New York, N.Y.

During the War there was an urgent need for a method to determine the adequacy of excision of traumatic neuromas. To accomplish this, various combinations of

stains were used on frozen sections; among them was one containing 2 gm. each of tannic acid, picric acid, aniline blue, and acid fuchsin in 100 cc. of water. Stains such as this are used in the textile industry for gross identification of fibers and in histology to distinguish varieties of connective tissue. For our present study on desoxyribonucleic acid, fibrinogen, and "fibrinoid" in tissue sections, the same stains are dissolved in saline solution instead of distilled water. Paraffin sections from routine formalin-fixed material may be used. If the concentrated formalin is diluted with salt solution instead of water, the results are clearer. However, better staining is obtained by fixing tissue in a mixture of equal parts of formalin, 95 per cent alcohol, glacial acetic acid, and physiologic saline solution with 100 mg. per cent calcium chloride in each 100 cc. solution. The sections are deparaffinized, brought into 1 per cent acetic acid in saline solution, immersed in the stain for 30 seconds, washed in 1 per cent acetic acid in saline solution, blotted, rinsed in tertiary butyl alcohol, blotted again, placed in xylol, and mounted in balsam.

The normal nuclei contain a few fine red granules or no red component, more red granules being present in nuclei near areas of malignancy. The chromosomes in mitosis stain a brilliant red contrasting with the delicate blue fibers of the spindle. Red cytoplasmic inclusion bodies are present in tumor cells from glioblastomas. Ordinarily, collagen stains blue, but if traumatized or compressed it stains red. Frequently red fibers are found among the blue fibers, especially in "young" collagen. In the kidney, the basement membrane of the normal glomerulus is blue, the intercapillary glomerulosclerotic lesion stains blue while the glomerular lesion in lupus erythematosus stains red, as does fibrinoid. Liver bile capillary outlines are blue. Bile thrombi vary in color, being green, red, blue, or black, but the bile thrombi are usually of one color in an individual case. In frozen section of nerves, fresh myelin is bright red while the axis cylinder is blue. In normal vaginal smears, most of the cells stain blue with an occasional red stained cell. Desoxyribonucleic acid and fibrinogen stain red, while ribonucleic acid and fibrin stain blue. The stain can be applied to filter paper chromatographs, fibrinogen appearing as a red line or spot; in the blue albumin and gamma globulin spots, a red element is present.

GROWTH OF HUMAN TUMORS IN THE ANTERIOR CHAMBER OF THE EYE IN GUINEA-PIGS TREATED WITH CORTISONE. Abe Towbin,* Ohio State University, Columbus, Ohio.

Malignant tumors from 94 patients were transplanted to the anterior chambers of the eyes of cortisone treated guinea-pigs. A number of transplantation techniques have been devised by various investigators in an effort to provide a feasible method of growing human tumors in laboratory animals. Two developments are of particular significance: the use of the anterior chamber technique and the administration of cortisone. In the present study these two procedures were combined in an effort to obtain optional transplantation conditions and thus to increase the percentage of successful transplantations.

The anterior chamber of the eye is a favorable site for tumor transplantation because it provides a milieu which tends to exhibit less foreign body reaction than other transplantation sites; in addition, direct observation of the transplant through the transparent cornea is possible. Cortisone has been shown to augment the heterotransplantability of skin, tumors, and other tissues. Heterologous tumor transplants are frequently destroyed by an overwhelming foreign body inflammatory reaction during the stage of nidation in the early period of transplantation. Cortisone, known to inhibit certain inflammatory processes, was used in the present investigation in an effort to reduce post-transplantation inflammation.

In a previous study, 100 clinically malignant human tumors were transplanted to untreated guinea-pigs; the transplanted tumor tissue showed definite growth in 2 cases. In the present study, in the cortisone treated animals the growth of anterior chamber transplants was significantly enhanced, initiation of transplant growth oc-

curred earlier, post-transplantation inflammation was reduced, and transplants of human tumors showed growth in a greater percentage and in greater volume.

THE COMPARATIVE PATHOLOGY OF ANDROBLASTOMA.† Nancy Warner, Nathan B. Friedman,* and Francis Masin, Cedars of Lebanon Hospital and University of Southern California, Los Angeles, Calif.

Destruction of the left ovaries of fowls is known to result in certain changes suggesting masculinization. The tumor-like proliferation of the right gonad which results in these animals resembles certain masculinizing ovarian tumors in women which have been described as arrhenoblastoma or androblastoma. Studies of the hormonal excretion of these birds will be presented. Comparisons will be made with the changes found in the ovaries of aged rats and in the gonads of rats transplanted to the spleen and thymus of castrate animals.

HISTOCHEMISTRY OF CHICKEN GONADS AND THEIR TISSUE CULTURES. Albert G. Smith,* Duke University School of Medicine, Durham, N.C.

Tissue culture completely changes the morphologic features of most cells from their usual forms as observed in ordinary histologic preparations, making the identification of cells difficult. The purpose of this study is to identify cells in the new growth zone of chicken gonadal tissue cultures by comparing their histochemical reactions to histochemical reactions of cells in gonadal tissues and to evaluate the facultative change in enzymatic processes that may take place when cells are cultured. Tests selected for this comparison are those for alkaline and acid phosphatase, esterase, ribonucleic acid, desoxyribonucleic acid, and the periodic acid-Schiff, Sudan red, and Sudan black reactions.

The alkaline phosphatase reaction was chiefly of help in highlighting supportive tissue, but few cells in 3-day cultures contained alkaline phosphatase, probably indicating that connective tissue did not compose much of the new growth zone of tissue cultures. The most valuable stains in identification of germ cells and peritoneal cells in cultures of embryo gonads were the acid phosphatase, esterase, and ribonucleic acid reactions. These three reactions were greatest in germ cells and in embryonal peritoneal cells in both tissues and tissue cultures. The periodic acid-Schiff and fat stains mirrored degeneration of cells in tissue cultures, although by the Sudan black stain it was possible to demonstrate formol-fixed lipid in the characteristic perinuclear mitochondrial caps of both tissue and tissue culture germ cells. The histochemical reactions of cells in tissue culture were less pronounced in general than those in the tissues. This decrease in reaction density is, in part, a real quantitative diminution and, in part, a decrease in density because of marked spreading out of cells in tissue culture.

FURTHER STUDIES OF THE PATHOGENESIS OF PULMONARY ADENOMAS IN MICE. Stanfield Rogers,* Duke University School of Medicine, Durham, N.C.

Work in this laboratory is concerned primarily in studying the mechanisms through which cells become neoplastic. Using *in vitro* systems of exposure, both nitrogen mustard and 2537 Å ultraviolet irradiation have been found to act directly upon the pulmonary cells in their initiation of neoplastic change. Urethane on the other hand has been found to act through an intermediary substance and not to be carcinogenic as such. *In vivo* studies have shown that substances that are used in, or influence, nucleic acid synthesis profoundly alter the relative number of tumors initiated by a single injection of urethane in intact mice. Orotic acid, a pyrimidine precursor, acts roughly as a competitive inhibitor of the carcinogenic action of urethane *in vivo*. In this report it will be shown that orotic acid also reduces the number of these tumors brought about by methylcholanthrene, *in vivo*. On the other hand, orotic acid alone,

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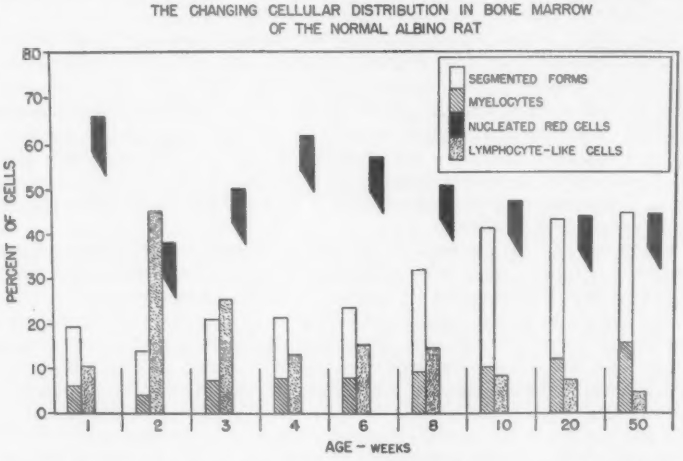
given in the drinking water over a period of many months, has itself a carcinogenic influence. In addition, it will be shown that in relatively high concentration it actually synergizes the carcinogenic influence of carcinogenic intermediate appearing in serum following urethane injection upon *in vitro* testing. In low concentrations the results are yet not wholly clear but in general appear inhibitory. The interrelations of these findings will be discussed.

THE CHANGING CELLULAR DISTRIBUTION IN BONE MARROW OF THE NORMAL ALBINO RAT BETWEEN 1 AND 50 WEEKS OF AGE.† Charles Harris and William T. Burke, Temple University Medical School, Philadelphia, Pa.

The cellular distribution of bone marrow of the albino rat changes during the first year of life according to the schema illustrated. Each value depicted represents the average of the differential counts of 1,000 cells in each of 10 rats. Samples of glutinous marrow were painted on slides with a brush and no hemodilution occurred.




Two periods occur when the cellular composition undergoes considerable change. In both instances, this change is related to the morphologic and numerical alterations in a distinctive mononuclear cell that resembles a small lymphocyte, and which normally accounts for 5 to 10 per cent of the marrow cells of rats up to 1 year of age. At 2 weeks of age, this cell, having undergone variation in size so that all diameters between about 12 $\mu$ and 20 $\mu$ are represented, comprises 40 per cent of marrow cells. As the cell increases in size, the chromatin becomes more lace-like and the cell cannot easily be differentiated from blast forms. This reaction subsides during the third week of life and the marrow differential count of animals 4 weeks of age is similar to that of animals at 1 week. The differential count then remains relatively constant until 6 weeks of age when the lymphocyte-like cell undergoes transformation of another type. Nuclear chromatin becomes less hyaline and more clumped; azurophilic granules appear, and benzidine positivity develops; a hole appears in the nucleus and the cell can no longer be differentiated from the ring-form of the polymorphonuclear leukocyte, a form common to the rat. It is this transformation that accounts for the increase of granulocytes of rat marrow from 20 to 40 per cent of total cells, in animals 6 to 10 weeks of age.

† Supported in part by research grant C–2206 from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

THE PATHOLOGY OF THE SPLEEN IN CONGENITAL HEMOLYTIC ANEMIA. Olin K. Wiland and Edward B. Smith,* University of Indiana Medical Center, Indianapolis, Ind.

There are numerous reports in the literature regarding the morphology of the spleen in congenital hemolytic anemia, but there is considerable disagreement concerning many of the individual features, and the distinctive over-all pattern has not been emphasized. The surgically removed spleens from 25 cases of congenital hemolytic anemia were studied in detail and were compared with the spleens from 113 control cases. The control group consisted of 64 sudden accidental death cases, 24 brain tumor cases, and 25 pneumonia cases. These three groups of cases were classified according to the predominant lesion or disease found at necropsy.

In the congenital hemolytic anemia cases the average enlargement of the spleen was five times the normal weight. There was massive engorgement of erythrocytes in the interstitial (extra-sinusoidal) tissue with compressed and relatively empty sinusoids. The endothelium of the sinusoids was quite prominent. The lymphoid follicles were widely separated and diminished in concentration. Hemosiderosis was a constant finding and was not related to the administration of blood transfusions. Siderotic nodules were uncommon in the first decade, but they were frequent in the succeeding decades. Erythropoiesis was present to some degree in all of these cases. These morphologic alterations in the spleen are not outstanding individually, but in combination they form a pattern which is characteristic of the disease.

STUDIES OF INTERSTITIAL RADIOLUTECIUM ($Lu^{177}$) IN THE DOG. William M. Christopherson, Harold F. Berg, Granuil C. Kyker, and Marshall Brucer, University of Louisville School of Medicine, Louisville, Ky., and Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.

The ease with which radioactive rare earths can be coupled with other chemicals, traced, and located with precision within the body, makes them adaptable for a wide range of studies. Lutecium$^{177}$ is primarily a low energy beta particle emitter, so that most of the radiation energy is absorbed by the tissue very close to the point of deposition. Like other rare earths, hydrolysis of ionic lutecium approaches completion before reaching the pH of blood. The hydroxide is insoluble, and its rapid removal from the blood and deposition largely in the liver and spleen suggest colloidal properties. Animal experiments were designed to establish whether or not radiolutecium behaves as a colloid when injected into the mammary gland of the dog. The study includes the degree and rapidity of lymph node concentration; the intranodal distribution patterns of the isotope; the general dosage required to produce irradiation changes in lymph nodes; the general body effect; and the tolerance of the tissues to the irradiation at the site of injection.

Radioassays were made on the various organs of the body. Histologic sections were examined and appropriate radioautograms were made throughout the study. Nineteen female dogs were studied with varying time dosage relationships. The study of the reticulo-endothelial system was further pursued to determine the effect of various complexing agents, as well as the specific activity as influenced by the amount and type of carrier used.

THE CORRELATION OF THE LETHAL EFFECTS OF X-IRRADIATION ON MOUSE POPULATIONS AND THE RATE OF GROWTH OF THE INDIVIDUAL ANIMAL AT THE TIME OF IRRADIATION. Jules Gordon, John McClaren, Audrey Komrad, and Stanfield Rogers,* Duke University School of Medicine. Durham, N.C.

Mouse populations of the same age, weight, sex, and inbred strain vary individually over a broad range in their resistance to the lethal effects of x-irradiation at a given time. The level of this variation has been used widely in testing the influences of irradiation under experimentally varied conditions, and is known, of course, as the $LD_{50}$. As there is such a broad range between the susceptibilities of matched

normal mice to these lethal effects it seemed that useful information might be obtained should the biologic influences responsible for the existence of an $LD_{50}$ under "normal" conditions be brought to light.

It is the general experience that rapidly growing cells are the most susceptible to the lethal effects of x-irradiation. It is also well known that individual animals have a diurnal cycle of cell growth. Our experiments show that there is a correlation between the mitotic rate of individual mice of the same strain, weight, age, and sex and their individual survival following high doses of x-irradiation, the individual animals growing most rapidly at the time of exposure being the most likely to die. Fasting mice for 48 hours prior to irradiation, to reduce their mitotic rate, increases their susceptibility to the lethal effects of irradiation. That this result is not incompatible with, but actually confirms, the previous correlation with high mitotic rate is shown by the fact that such fasting increases the number of cells in late interphase, just prior to cell division—a cellular physiologic phase known in simple biologic systems to be most susceptible to the lethal effects of irradiation. The significance of these findings will be discussed.

RÔLE OF VASCULAR INJURY IN LATE RADIATION LESIONS. George Brecher* and Victor P. Bond, National Institutes of Health, Bethesda, Md., and Naval Radiological Defense Laboratory, San Francisco, Calif.

The rôle of vascular injury in chronic irradiation lesions was studied in rats. Lesions of kidneys, vertebrae, and spinal cords were produced by single exposures to a narrow deuteron beam, ½″ in diameter, of 1800 to 2600 rep. The animals were killed 3 to 7 months after irradiation. The affected parts of the renal cortex and medulla were shrunken, with extensive destruction of tubules, patchy hemosiderin deposition, and minimal fibrosis. Glomeruli appeared nearly normal. Demyelinization of spinal nerve roots was nearly complete, in contrast to the unchanged myelin in the cord. Necrosis and resorption of bone were evident and the marrow was replaced by loose connective tissue. The spinal musculature was partly replaced by fat. Regeneration of muscle occurred only rarely. Vascular lesions consisted, in various combinations, of fibrinoid deposition in the intima, hyalinization of the intima or media or both, and occasional adventitial proliferation. Arterioles and venules were primarily affected. However, the borders of the sharply circumscribed lesions of the kidney and spine, including cord, bone and muscle, appeared to be determined by the path of the beam and not by the distribution of vascular lesions.

DEMYELINIZATION INDUCED IN THE BRAINS OF MONKEYS BY MEANS OF FAST NEUTRONS: PATHOGENESIS OF THE LESION AND COMPARISON WITH THOSE OF DISSEMINATED ENCEPHALOMYELITIS IN HUMAN BEINGS. F. Stephen Vogel* and John E. Pickering, New York Hospital-Cornell Medical Center, New York, N.Y., and School of Aviation Medicine, Randolph Air Force Base, Texas.

During studies on the biologic effects of neutrons it was observed that demyelinization was regularly present in the brains of monkeys sacrificed more than a year after exposure of the ocular and peri-ocular regions to 850 rep. of 14 mev. radiation from the Cockcroft-Walton accelerator. Histologic examinations threw light on the pathogenesis of the changes. There was atrophy of the rostral portions of the brain in all 6 monkeys sacrificed 14 to 22 months after exposure, but no gross or microscopic alteration in the central nervous system of one animal examined 2 months after radiation under identical conditions, or in those of 5 normal animals used for control purposes. The degrees of atrophy and the extent of the histologic changes were related directly to the survival period after radiation. The white matter of the frontal lobes of animals sacrificed 14 and 17 months after exposure contained small focal lesions characterized by swelling, fragmentation, and loss of myelin sheaths. These regions and the rostral portions of the parietal lobes of animals sacrificed a few months

later were almost devoid of myelin. Cajal's silver nitrate stain regularly disclosed dense plexuses of axis cylinders. Many of these crossed large areas of demyelinization and only a few were beaded and fragmented. The tissues in and about the large areas of demyelinization, when stained with Hortega's silver carbonate method, were found to contain multitudes of fibrillary astrocytes, an increased number of microglia, and a moderately decreased number of oligodendroglia. The oligodendroglial cells showed only minor cytologic changes, notably a loss and shortening of the neuroglial processes. The nerve cells rarely appeared altered in tissues stained by Nissl's and Cajal's silver nitrate methods. Inflammation was regularly scant. The walls of the cerebral, meningeal, and choroidal blood vessels were generally unaltered; only a few medium-sized arteries and veins showed endothelial proliferation and sclerosis.

The findings make it plain that the radiation with neutrons brought about, after some months, primary focal degeneration of myelin which became more marked with the passage of time and led to degeneration of the axis cylinders, marked astrocytic gliosis, proliferation of microglia, and minor cytologic alterations in the oligodendroglia, the whole process occurring essentially without inflammation. In these particulars the lesion resembles closely those that characterize the two common forms of disseminated encephalomyelitis in human beings, namely, multiple sclerosis and Schilder's disease.

DIFFERENTIAL EFFECTS ON CELLULAR DIVISION AND DIFFERENTIATION.† Nathan B. Friedman,* Robert E. Hoyt,* and Eileen Drutz, Cedars of Lebanon Hospital and University of Southern California, Los Angeles, Calif.

Previous work has shown that irradiation and certain chemotherapeutic agents do not interfere with differentiation of intestinal epithelium although they do inhibit division. Other chemical agents affect both division and differentiation. These studies have been extended to include other biologic systems. Certain effects in transplantable tumors and bacterial cultures suggest that the dichotomy between division and differentiation may represent a general phenomenon.

FIXATION OF RADIOACTIVE SULFATE BY CARCINOMAS OF THE COLON AND OF THE BREAST.‡ Raymond G. Gottschalk,* Howard N. Beers, and Benedict J. Duffy, Jr., Veterans Administration Center, Martinsburg, W. Va., George Washington University School of Medicine and Georgetown University Medical School, Washington, D.C.

Selective uptake of $S^{35}$-labeled sulfate by mucin-secreting carcinomas was anticipated because of the presence of sulfated mucopolysaccharides both in the epithelial mucin and in the proliferating connection tissue stroma. Tracer amounts (1 to 2 mc.) of $Na_2S^{35}O_4$ were administered intravenously to 6 patients with carcinoma of the colon or rectum and to 3 patients with breast carcinoma. Specimens obtained during surgery, 14 to 66 hours later, were analyzed for total $S^{35}$ content and used for radioautographs. In each patient the uptake of isotope in the neoplasm exceeded that in the other samples obtained at the same time, generally skin, adipose tissue, muscle, intestine, and plasma. The concentration of $S^{35}$ in the carcinomas of the colon was 1.6 to 2.9 times that in the wall of the large intestine, but was not greater than that in the isolated colonic mucosa, because of the large uptake by mucus-secreting cells soon after injection. The $S^{35}$ concentration in carcinomas of the colon was 5 to 10 times that in normal skin (epidermis and dermis); the concentration in breast carcinomas was about twice that in skin. Radioautographs showed only minimal uptake in all tissues except the neoplasms and intestinal epithelium. Uptake in the tumors occurred consistently in the connective tissue stroma, also to a greater or lesser degree in the neoplastic epithelium, and in a spotty fashion in the tumor mucin, prob-

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ably indicating sites of recent production. Further studies are needed to indicate how long $S^{35}$ is retained in these neoplasms. The selective concentration of the radioactive isotopes in these tumors may be of help during surgery to ascertain their extension for complete removal.

A Co-Carcinogenic Effect of Certain Nucleotides Upon Irradiated Hematopoietic Tissues. William E. Smith,* Milton Friedman, and Anna M. Slicher, Post-Graduate Medical School, New York University, New York, N.Y.

Young female mice of the C strain were divided into five groups of 30 animals each and observed throughout their lives. In an untreated control group, leukemia has eventually developed in 7 animals, the deaths from this disease occurring between 300 and 500 days after start of the experiment. In a group exposed to 275 r. whole body irradiation, there were 4 cases of leukemia, occurring between 425 and 470 days. A group exposed to 275 r. and subsequently given repeated injections of 25 mg. of equal parts of uridylic and cytidylic acids in 0.5 cc. 4 per cent disodium phosphate buffer yielded 12 cases of leukemia between 148 and 415 days. Groups of unirradiated animals treated with the buffer alone or the solution of uridylic and cytidylic acids yielded only 2 cases and 1 case of leukemia, respectively.

The results may be more clearly visualized by considering findings at a single point in the tests, such as the 400th day. At that time, no leukemias had occurred in the group exposed merely to 275 r., and only one leukemic death in each non-irradiated group, i.e., the untreated controls, the group given buffer, and the group given the nucleotides, uridylic and cytidylic acids; whereas 10 animals had by that time died of leukemia in the group that had been given irradiation plus nucleotides.

The findings indicate that a dose of irradiation, insufficient in itself to increase the frequency of leukemia, conveyed a liability to this disease that became manifest upon subsequent exposure to the nucleotides employed. These nucleotides exhibited no leukemogenic action in animals not previously exposed to irradiation. The reduction of leukemia incidence in unirradiated animals, treated with these nucleotides, to a level below that observed in an untreated control group suggests an inhibitory action of these compounds upon the spontaneous disease; but this effect may be due merely to impairment of health caused by repeated injections since a comparable reduction in the frequency of leukemia was observed in the group treated merely with the buffer employed to dissolve the nucleotides.

Morphologic Changes Produced in Adult Trichinae by Gamma Radiation. S. E. Gould,* H. J. Gomberg, J. B. Villella, and S. Hertz, Wayne County General Hospital, Eloise, Mich.; Wayne University College of Medicine, Detroit, Mich., and University of Michigan, Ann Arbor, Mich.

Measurements were made of the length and maximum width of the body and of the gonads of male and female trichinellae recovered from the intestine of white rats 3 and 6 days after infection with larvae irradiated with 1,000 to 15,000 r. cobalt-60, and 1,000 to 4,000 r. x-ray. In addition, intestinal trichinae, in the living and in the fixed state, were examined for evidence of morphologic changes at various intervals from 24 to 144 hours after infection of white rats with larvae exposed to 2,000 to 4,000 r. x-ray.

The higher doses of cobalt-60 (12,000 to 15,000 r.) and of x-ray (3,000 to 4,000 r.) produced decreases in size, particularly of body length. Six days after exposure to 2,000 r. x-ray, only a small proportion of the females were inseminated; after 3,000 r., only an occasional female was inseminated and many of the egg cells were undergoing degeneration; and after 4,000 r., practically no females were inseminated and there was evidence of considerable degeneration of egg cells. A dose of 4,000 r., was followed by stunting of growth of males and females, extensive degeneration and shrinkage of the ovaries, and thickening and nodular swelling of the cuticle of females.

It seems likely that granular degeneration and disintegration of the egg cells was a factor in causing obstruction of the vagina and vulva and in some instances may have prevented insemination.

ACUTE ARTHRITIC LESIONS IN GUINEA-PIGS INDUCED BY BACTERIAL PRODUCTS. Russell S. Jones* and Yolande Carter, University of Utah College of Medicine, Salt Lake City, Utah.

Proliferation of synovial cells and formation of exudate, as previously reported by the authors, have been produced by the intravenous injection of whole, killed *Klebsiella pneumoniae* and crude protein and polysaccharide fractions therefrom. In addition, small (0.25 mg.) quantities of the somatic antigen of *Shigella paradysenteriae* induced these joint lesions. The first histologic evidences appeared within 24 hours after a single intravenous injection. The severity of the reaction increased with repeated injections but the lethal qualities of some of these bacterial fractions did not necessarily parallel the arthropathic properties. Bacterial fractions, labelled with $C^{14}$ by biosynthesis from 1-$C^{14}$ acetate, rapidly entered the joint space. The material remained for some days at this site and also appeared within the synovial membrane upon autoradiographic studies. Because of the rapidity of development of the lesions, antibody would not appear to be involved in the synovial region. In the guinea-pig, a relatively high concentration of the $C^{14}$ label is found within the adrenal cortex. The exact nature of the compound incorporated into the adrenal cortex has not been determined, but it is apparently stored here while the $C^{14}$ polysaccharide disappears from the spleen and liver. The histologic changes in the joints are similar to those in scurvy but the vitamin C concentration of adrenal gland, liver, and spleen is not significantly altered while joint lesions are still evident.

THE MORPHOLOGY OF ANTIBODY-PRODUCING LYMPH NODE AND PERITONEAL CELLS TRANSFERRED TO IMMUNOLOGICALLY INERT RECIPIENTS. James C. Roberts, Jr., and Frank J. Dixon,* University of Pittsburgh School of Medicine, Pittsburgh, Pa.

When lymph node cells from donor rabbits, immunized previously to bovine serum albumin, were transferred subcutaneously to x-radiated, immunologically inert recipient rabbits, and the recipients then challenged with this antigen, a secondary immune response was observed in the recipients. Immunochemical evidence indicated that the transferred donor cells were the sources of the immune responses observed. These cells synthesized amounts of antibody globulin nearly equal to their wet weight during the first 8 days after transfer. Recipient injection sites, obtained from 1 hour to 9 days after transfer, were studied. Killed lymph node cells also were transferred and these injection sites studied.

At the time of transfer, the lymph node cell suspensions consisted of about 85 per cent lymphocytes, 3 per cent plasma cells, and 10 per cent reticulo-endothelial and stem cells. One day after the transfer of live cell suspensions, the edematous injection sites contained readily visible well preserved donor cells and a few cells of the recipient's mesenchyme. At 2 days there was pyknosis of some of the transferred lymphocytes, and a granulocytic host response. From 3 to 6 days after transfer the number of recognizable lymphocytes became smaller and plasma cells developed in aggregates near the peripheries of the injection sites. Also during this period, the granulocytes diminished, foreign body and sarcolemmic giant cells developed, and fibroblastic activity became prominent.

When lymph node cells killed by heat, cold, or x-radiation, or alive but which failed, for unknown reasons, to make an immune response, were transferred, almost similar evolution of the injection sites occurred. However, there was an earlier appearance and increase of fibrous tissue, and no plasma cell aggregates were observed. From this, it seems likely that the plasma cells found after transfer of living cells came from the transferred lymph node cells rather than the host. The develop-

ment of plasma cells after transfer of lymph node cells correlated well with the antibody response of the transferred cells. When a number of sites obtained 8 to 10 days after transfer were studied, it was possible to predict almost all transfers resulting in a good immune response, and three fourths of the transfers resulting in a poor or no immune response.

Similar studies with peritoneal cells are in progress and will be discussed.

POTENTIATING EFFECTS OF ENDOGENOUS CORTICOIDS ON HUMAN ADRENOCORTICAL INFECTION AND NECROSIS. J. K. Frenkel,* University of Kansas School of Medicine, Kansas City, Kans.

It was observed in hamsters experimentally infected with the obligate intracellular protozoan, Besnoitia, that adrenal necrosis occurred during the course of chronic infection. Besnoitia organisms proliferated actively in the adrenal glands and necrosis was brought about by the destruction of parasitized cells. Meanwhile, there was little or no demonstrable proliferation of organisms in the other organs of the same host. It was shown in the acute infection that the peculiar susceptibility of the adrenal cortex was reversed following surgical hypophysectomy as well as after the administration of cortisone, both of which produced adrenal cortical atrophy, presumably due to lack of adrenocorticotrophic hormone (ACTH). The administration of ACTH to hypophysectomized hamsters restored a measure of susceptibility of the adrenal glands. During chronic infection, the administration of corticoids in pharmacologic doses led to a recurrence of proliferative activity by Besnoitia, and frequently the tissues surrounding the injection sites of cortisone acetate were parasitized. Although parasitization of the adrenal gland appeared to depend on a supply of endogenous or exogenous ACTH, it is more probable that the steroids produced by the adrenal cortical cells predisposed to their parasitization, since subcutaneous injection of similar steroids, but not of ACTH, predisposed to parasitization of the injection sites.

The question arose whether this experimental infection of the adrenal gland is pathogenetically analogous to adrenal necrosis in man. Consequently, a number of cases of Addison's disease due to tuberculosis, histoplasmosis, and coccidioidomycosis, were re-examined and the tissue reactions in the adrenal cortex were compared to those elsewhere in the same host. It was found that the adrenal glands showed extensive lesions, compared to the moderate or slight involvement of other organs. The tissue reaction in the adrenal glands suggested that certain cortical hormones were active here. In tuberculosis this reaction was characterized by a predominantly necrotizing process with a tendency to confluence, surrounded by a narrow rim of lymphocytes and mononuclear cells; only rarely was this accompanied by a few giant cells and a narrow zone of epithelioid reaction. In the other involved organs epithelioid reaction was prominent. Histoplasmosis of the adrenal glands consisted of a necrotizing lesion, unaccompanied by the mononuclear and fibroblastic reaction which was observed in other infected tissues. Coccidioidomycosis of the adrenal glands gave rise to necrosis and to slight epithelioid reaction, which was much less well developed than in the lungs and meninges of the same patient. More Histoplasma and Coccidioides organisms were present in the lesions of the adrenal glands than in those of the other organs.

It was observed in a case of histoplasmosis that a hormonal factor from the adrenal gland could impart increased susceptibility to another organ. Drainage via the central adrenal vein had become obstructed by necrosis, and blood from the few remaining adrenal cortical cells drained via the liver to which the right adrenal gland had become adherent. Only in the right lobe of the liver, adjacent to the adherent adrenal gland, did Histoplasma multiply actively. The remaining liver was free of lesions although several blood cultures had been positive.

It has been found repeatedly in man and in animals that cortisone and some of its congeners facilitate the proliferation of certain microorganisms. It has also been discussed by Lurie and Zappasodi that cortisone retards the maturation of epithelioid

cells and of the caseous process. The predominant findings of necrotizing lesions rather than of epithelioid reaction in the adrenal glands involved with tuberculosis, histoplasmosis, and coccidioidomycosis can be ascribed to the anti-inflammatory effects of local corticoids. The selective involvement of the adrenal glands and the increased number of microorganisms found therein support the hypothesis that microbial proliferation in the adrenal glands of an otherwise relatively immune host depends on the local immunity-depressing effects of the corticoids elaborated in these adrenal glands.

THE EFFECTS OF CORTISONE AND CHEMICAL INFLAMMATION ON RHIZOPUS ORYZAE INFECTION. Heinz Bauer, George L. Wallace, Jr., and Walter H. Sheldon,* Emory University School of Medicine, Emory University, Ga.

The fungus *Rhizopus oryzae*, because of its clearly defined differences in behavior in metabolically normal and in abnormal hosts, is a unique test agent with which to study the effects of metabolism on infection. The fungus, isolated post-mortem from a diabetic patient with cerebral mucormycosis, produces in rabbits with alloxan diabetes extensive nasal, cerebral, and pulmonary lesions in which the polymorphonuclear leukocytes show degenerative changes. Non-diabetic animals show only rare insignificant lesions at the inoculation site. Alterations of host metabolism, therefore, are essential to change *R. oryzae* from a saprophytic to a pathogenic organism. To define these metabolic factors the following experiments were undertaken. Because of the known decrease in host resistance after cortisone, rabbits treated with this hormone were inoculated with the fungus. In other rabbits, the site of a chemically induced inflammation in the nose was secondarily inoculated with fungus, because necropsy findings in man suggested that pre-existing inflammation might produce local conditions suitable for the fungus infection.

Cortisone (10 mg.) was given daily to 17 rabbits for 2 to 21 days, followed by intranasal instillation of a spore suspension of *R. oryzae*. All animals were sacrificed 48 hours after inoculation while still on cortisone. No lesions resembling those in diabetic rabbits were found. Minute focal nasal lesions with rare degenerating spores were present in 9 animals, 2 of which showed rare minimal pulmonary inflammatory foci with few degenerating spores. In these lesions, the leukocytes, although perhaps quantitatively decreased, appeared normal. Slightly more marked nasal lesions with rare mycelia occurred in another animal. No lesions were present in the remaining 7 rabbits. Marked cortisone effect was seen in all animals and fungus cultures of nose and/or lung were positive in every instance.

Croton oil (0.3 cc.) was instilled intranasally in 18 rabbits followed 48 or 96 hours later by fungus inoculation. The animals were sacrificed 48 or 96 hours after inoculation. Extensive inflammatory nasal lesions with bacteria were present in all animals, 11 of which also showed pulmonary lesions. These closely resembled the lesions in 8 controls receiving croton oil followed by intranasal saline solution, and showed a marked response with normal leukocytes. No fungus proliferation had occurred and spores were found with difficulty while fungus cultures of nose and/or lung were positive in all but 3 animals.

Our findings show that cortisone in the amount given does not produce conditions suitable for the proliferation of *R. oryzae* in tissues, although the increased incidence of minute focal lesions resembling those in normal animals indicates an altered host response. The scarcity of demonstrable spores and the absence of fungus growth in chemically induced inflammatory lesions secondarily infected by bacteria suggest that a pre-existing focus inhibits rather than favors infection with this organism. Differences in the character of the leukocytic response in these and the previously reported studies in diabetic rabbits suggest that the metabolic alterations essential for this fungus infection may act through their effect on the polymorphonuclear leukocyte.

THE COLORATION OF ACIDIC CARBOHYDRATES OF BACTERIA AND FUNGI IN TISSUE SECTIONS WITH SPECIAL REFERENCE TO CAPSULES OF CRYPTOCOCCUS NEOFORMANS, PNEUMOCOCCI, AND STAPHYLOCOCCI.† Robert W. Mowry* and Charles H. Winkler, University of Alabama Medical Center, Birmingham, Ala.

Despite the great importance attributed to the carbohydrate capsules of pneumococci, little or no regard has been paid to capsule staining in histologic studies of infectious processes, whether due to pneumococci or other agents. While the value of the periodic acid-Schiff reaction for coloring the cell bodies of pathogenic fungi is well known, cytochemical methods for acidic carbohydrates have so far been little used. Novelli did publish a method for staining bacterial capsules with Alcian blue 8GS. One of us (R.W.M.) found in other studies that Alcian blue stained acidic carbohydrates much better and more selectively when a lower pH and dye concentration were used. This and some other improved methods have been tested by us as stains for capsular carbohydrates. Paraffin sections were hydrated and stained by the following procedures:

1) *Alcian Blue Stain (AB).* Prepare 0.1% AB in 3% acetic acid, filter, and add a crystal of thymol. Stain for 30 minutes in AB. After rinsing, counterstain with saturated aqueous picric acid. If a nuclear stain is desired, perform the Feulgen reaction. Dehydrate, clear, and mount. Result: Acidic substances, chiefly carbohydrates, are colored bright blue. Picric colors the background yellow. The Feulgen reaction colors nuclei magenta.

2) *Alcian Blue-Periodic Acid-Schiff Combination (AB-PAS).* Follow the plain AB stain with the routine PAS procedure, omitting hematoxylin. Result: Strongly acidic substances, blue; 1,2-glycols, magenta; and substances with both, purple.

3) *Toluidine Blue O.* Stain 5 minutes in a 0.1% solution. Rinse in water and quickly dehydrate in 95% and absolute alcohols. Allow section to *dry* at room temperature. (Cooling during drying apparently causes the condensation of trace moisture, favorable to metachromasia.) Then clear in xylene and mount. Result: Strongly acidic substances, presumably carbohydrates, are red, i.e., metachromatic; while nucleic acids are usually blue, i.e., orthochromatic. Use more dilute dye if staining is too heavy.

Tissue sections, known to contain gram-positive organisms, from 10 cases of proved infection with pneumococci and staphylococci were stained and compared. In the majority the organisms were stained and often showed well defined rings of apparent acidic carbohydrate strongly suggestive of capsules. Although staphylococci are not usually regarded as bearing capsules, Lyons and a few other workers have described them.

The stains were applied also to numerous mycotic lesions, some of which were supplied us by the Armed Forces Institute of Pathology. The thick shaggy capsules of *Cryptococcus neoformans* as seen in tissue sections are brilliantly colored by Alcian blue and are red after toluidine blue. After capsule-staining, *C. neoformans* is so morphologically distinctive that other budding fungi are easily excluded. Although mucinous capsules are often absent in the smallest or youngest organisms, encapsulated larger forms are always plentiful. The AB-PAS stain gives the most complete demonstration of organisms. The capsules are fairly thick but not so wide as indicated in India-ink preparations. In some cases of *Blastomyces dermatitidis,* a minority of organisms show a faintly to moderately colored smooth rim of acidic material, possibly capsule. In addition, the AB-PAS stain will show central masses of PAS-positive material in *B. dermatitidis* that are not usually seen in *C. neoformans.* Definite capsules of acidic carbohydrate were not seen in specimens of histoplasmosis and candidiasis. In tissue from mice infected with *Sporotrichum schenki,* cell bodies appeared to contain acidic material but capsules were not plainly present.

Cytochemical reactions for acidic carbohydrates permit the study of certain

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microbial capsules and variations in capsulation in infected tissues. Correlation of chemical knowledge about capsular carbohydrates with staining properties will be of value in carbohydrate cytochemistry. The staining of acidic carbohydrate(s) in the capsules of *C. neoformans* deserves wider use as an aid in recognition.

THE PATHOLOGIC PICTURE PRODUCED BY HUMAN INFECTION WITH MYCOBACTERIUM KANSASII (THE "YELLOW BACILLUS").† Ann Pollak, University of Kansas Medical Center, Kansas City, Kans.

Seventeen cases of human infection with an atypical acid-fast organism have been reported previously (Buhler and Pollak, 1953; Wood, Buhler, and Pollak, 1956). All attempts to recover typical tubercle bacilli from these patients were unsuccessful, including direct inoculation of infected material into guinea-pigs. Instead, an organism, previously called the "yellow bacillus," was found in each of these cases. It grows rapidly on a wide variety of media and produces a smooth yellow colony. It fails to produce progressive disease in guinea-pigs, but regularly does so in hamsters. Recently, this organism has been named *Mycobacterium kansasii* (Hauduroy, 1955).

The pathologic material that forms the basis of this report consists of 3 necropsies and 12 surgical specimens obtained from 9 patients. Grossly, when the disease is confined to the lungs, the pathologic findings are indistinguishable from tuberculosis. Cavities form in the apical portion of the lungs, with or without diffuse pneumonic infiltration. The disseminated disease presents a dramatic picture at necropsy. There are numerous large caseous foci in the lungs, spleen, and lymph nodes with smaller nodules elsewhere. The microscopic resemblance to tuberculosis is less striking in both the localized and disseminated forms. There are caseous foci surrounded by a polymorphic type of infiltration consisting of macrophages, lymphocytes, and plasma cells, with occasional polymorphonuclear leukocytes and eosinophils. Giant cells and distinct tubercles are rare. In the localized disease, acid-fast organisms are few and hard to find, but in the disseminated disease, innumerable bacilli are found in the lesions. A pathologic picture similar to the disseminated disease can be produced in hamsters.

EFFECTS OF CHEMOTHERAPY ON CASEOUS LESIONS OF TUBERCULOSIS. Adolf Hochwald,* Ray Brook State Tuberculosis Hospital, Ray Brook, N.Y.

The present observations are based on a study of 150 resected specimens of pulmonary tuberculosis. The great majority were from successfully treated patients, success being defined as rapid improvement in the clinical and radiologic findings and disappearance of tubercle bacilli from the sputum. In the "successfully treated" cases resection was performed after the sputum had been negative, by culture, for at least 3 months, and after at least 6 months of combined chemotherapy. The findings show a distinct healing pattern which is illustrated by repeatedly observed changes.

The most frequent finding—for the purpose of this presentation called the "type lesion"—is a caseous focus surrounded by a sharply demarcated thin capsule of concentrically lamellated fibrous tissue without signs of activity in form of granulation tissue. On the outside of the capsule may be clusters of lymphocytes and of frequently atypical giant cells. Earlier stages contain a layer of granulation tissue interposed between necrotic center and fibrous wall. Further development of the "type lesion" takes place along two lines. Firstly, by fibrosis, which apparently begins from a segment of the surrounding wall and gradually organizes the caseous focus. The end stage, in smaller lesions, is a nodule of fibrohyalin surrounded by concentrically lamellated fibrous tissue. Secondly, by calcification. Here the end stage is a calcific nodule, or, in rare instances, partial or complete ossification.

In drug-resistant patients cavities resemble those of untreated cases. In several

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"successfully treated" cases the cavities were empty, their walls thin, smooth, and glistening. Microscopically, the wall consisted of lamellated fibrous tissue, similar to the capsule of solid lesions. The lumen might contain a few histiocytes or an occasional giant cell attached to the wall, or portions of the wall might be lined by columnar or stratified squamous epithelium.

Search for acid-fast organisms in sections sometimes revealed, in the "type lesion," large numbers of bacteria in the center of the caseation, but more frequently none at all. In more advanced lesions with appreciable fibrosis, bacteria were consistently absent.

THE HYDROPIC EFFECT OF SYNTHALIN-A ON PANCREATIC ALPHA CELLS OF THE GUINEA-PIG. W. E. Toreson,* University of California School of Medicine, San Francisco, Calif.

The response of the guinea-pig to injections of synthalin-A (decamethylene diguanidine) is similar biologically to the response of the rabbit. Temporary phases of hyperglycemia and profound hypoglycemia are followed by return of the blood sugar to normal levels. Albuminuria is present regularly, glucosuria occasionally, and ketonuria rarely. Necrosis of the proximal convoluted renal tubules is associated with severe azotemia. The liver cells at the periphery of the lobules exhibit severe fatty change. The pancreatic lesion is selective for alpha cells. Degranulation is evident within a few hours; it is followed by extreme hydropic swelling of the cytoplasm and subsequently by dissolution of the alpha cells. Animals that survive 72 hours and longer show cellular regeneration in the kidney, liver, and pancreatic islets. The hydropic alpha cell cytoplasm does not contain fat or glycogen.

CORRELATION OF ALPHA CELL MORPHOLOGY AND GLUCAGON CONTENT OF PANCREAS. S. A. Bencosme, S. S. Lazarus, E. Liepa, J. Frei, and S. Mariz, Queen's University, Kingston, Ont.

In an attempt to establish whether the alpha cell of the pancreas is the site of origin of pancreatic glucagon, several substances known to alter the number and the structure of alpha cells were tested. Histologic and cytologic procedures capable of defining precisely the morphologic state of the islets were used. The degree of change in the alpha cells was correlated with the hyperglycemic activity of pancreatic extracts in the cat. These results were compared with those obtained with extracts from the uncinate process of the pancreas of the dog, which we recently demonstrated to be devoid normally of alpha cells.

Marked lesions were observed in the alpha cells of cobalt treated guinea-pigs; but changes were not seen in the alpha cells of similarly treated dogs, rabbits, rats, or chickens. However, under other experimental conditions, one of us (S.S.L.) has produced alpha cell alterations in the rabbit and dog pancreas with cobalt. Synthalin-A did not alter the morphologic features of alpha cells in normal or in alloxan-diabetic dogs, but did produce some hydropic degeneration of alpha cells in normal and in alloxan-diabetic rabbits. I.P.T.D. (4-Aminobenzolsulfon-2-isopropyl-1-1,3,4-thiodiazol-5-imid) did not alter the alpha cells in dogs or in rats.

Almost complete lack of hyperglycemic activity was observed with pancreatic extracts whenever the alpha cells were absent, as in the uncinate process of the normal, the alloxanized, and the partial pancreatectomized dog pancreas; or when almost every alpha cell was severely damaged as in the cobalt treated guinea-pig pancreas with a 4-plus lesion. On the other hand, no significant change could be seen when 1, 2, or 3 plus alpha cell damage had been produced. These findings are interpreted as indicating that glucagon is produced by the alpha cell. However, almost complete absence of the alpha cells or of the alpha cell granules is necessary before qualitative methods of glucagon determination show diminution of this substance.

THE PREDILECTION OF PANCREATIC BETA CELLS FOR PIGMENT DEPOSITION IN HEMOCHROMATOSIS AND HEMOSIDEROSIS. Malcolm H. McGavran and W. Stanley Hartroft,* Washington University School of Medicine, St. Louis, Mo.

To the authors' knowledge, the cellular localization of hemosiderin in pancreatic islets has not been specified in previous reports. Paraffin blocks of 23 pancreases, 10 of idiopathic hemochromatosis and 13 from advanced transfusional hemosiderosis, were sectioned at 4 $\mu$. In addition to hematoxylin and eosin, they were stained by (a) Gomori's chrome alum hematoxylin-phloxine, (b) Gomori's aldehyde fuchsin as modified by Wilson, and (c) Turnbull-Perles's iron reaction. All islet cells were counted regardless of type. Alpha cells were identified in the chrome alum hematoxylin-phloxine stained sections as those in which the cytoplasm was filled with fine red granules. The number of identifiable alpha cells (i.e., only those containing stained granules), was expressed as a simple percentage of total islet parenchymal cells.

In the hemochromatic pancreases almost all beta cells contained pigment, whereas rarely did an identifiable alpha cell contain even minute amounts. The more pigment the less the beta granulation. Counts indicated an approximate average of 8 per cent alpha cells in these cases, with a range from 0.3 to 30 per cent. In the hemosiderotic pancreases again the pigment was predominantly within the beta cells. Alpha cells containing pigment, although relatively more numerous than in the hemochromatotic pancreases, were still very sparse. Counts indicated an average of approximately 16 per cent alpha cells, with a range of 2 to 42 per cent. The difference in the averages of 8 and 16 per cent is not statistically significant. The predisposition of pigmentation of the beta cells is definite. No hyalinized islets were found in these cases. The apparent decrease in alpha cell population is in contrast to that of adult patients with diabetes mellitus.

THE EFFECTS OF SODIUM RESTRICTION AND POTASSIUM EXCESS ON THE DOG ADRENAL GLAND: A POSSIBLE RELATION BETWEEN MORPHOLOGIC CHANGES AND SERUM ELECTROLYTE CONCENTRATION.† George J. Race, W. M. Nickey, Phillip S. Wolf, and Elmer J. Jordan, University of Texas Southwestern Medical School, Dallas, Texas.

Evidence has accumulated to indicate that each zone of the adrenal cortex is concerned with the secretion of different adrenal cortical hormones. It has been suggested that the zona glomerulosa is responsible for secretion of electrolyte regulating desoxycorticoid type of hormone. Previous studies by several authors on rats have shown that hypertrophy of the zona glomerulosa can be produced by hyponatremia or by hyperkalemia. The current study was designed to investigate similar stimuli in the dogs. Dogs were rendered hyponatremic by peritoneal dialysis with 5 per cent glucose in distilled water while their serum potassium levels were kept normal or increased by a dietary supplement of KCl. Serial serum sodium and potassium determinations were made with a flame photometer to determine the electrolyte status. Following sacrifice of the animals the combined adrenal weights were obtained and measurements of the zona glomerulosa were compared with those on adrenal glands of a group of control dogs.

Studies at this time show that the combined adrenal weights are increased in the dogs rendered hyponatremic. The zona glomerulosa of the adrenal glands of these dogs appears to be slightly hypertrophied. It is suggested that the minimal changes in the glomerulosa are possibly due to the decreased sodium:potassium ratio of the serum and that the disturbance of that ratio possibly stimulates electrolyte-regulating hormone production by the zona glomerulosa.

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SOME EFFECTS OF CASTRATION AND HORMONE THERAPY ON THE HYPOPHYSIS OF MEN WITH CARCINOMA OF THE PROSTATE. Agnes Burt Russfield, Massachusetts General Hospital, Boston, Mass.

The hypophyses of 33 patients with carcinoma of the prostate were studied histologically using the periodic acid-Schiff reaction. In patients who had received no hormone therapy, the hypophysis contained an increased percentage of hypertrophic amphophil cells similar to those found in women with untreated carcinoma of the breast. In some of these patients, hypophyseal weight was significantly increased. Castration alone tended to increase the percentage of amphophils. Estrogens, either alone or following castration, and cortisone tended to suppress the amphophils and increase the percentage of basophils. Prolonged hormone therapy appeared to decrease hypophyseal weight. These findings suggest that there may be some type of hypophyseal overactivity in patients with untreated carcinoma of the prostate and that the beneficial effects of estrogens and cortisone in this disease may be produced in part by hypophyseal suppression.

MULTIPLE, SIMULTANEOUSLY OCCURRING, ENDOCRINE TUMORS. L. J. McCormack,* P. G. Skillern, and S. O. Hoerr, Cleveland Clinic, Cleveland, Ohio.

Multiple, simultaneously occurring tumors of the various endocrine glands form an interesting and unusual group. It is our purpose to present 4 cases (2 diagnosed and treated clinically and 2 studied at necropsy) of this disorder.

The 2 clinical cases had given symptomatic and/or chemical evidences of hyperinsulinism and hyperparathyroidism. At surgery, both were found to have multiple islet cell tumors of gross and microscopic size. The cellular features of the adenomas were variable and only rarely was the intervening tissue between the cords of tumor cells hyalinized. Each showed numerous beta granulated islet cells within adjacent islands of Langerhans. One of these cases also had a marked anisonucleosus of the islet cells. Both of these cases became permanently diabetic following subtotal resection of the pancreas. At the time of subsequent surgical intervention, a parathyroid adenoma was found in one case; in the other "nodular hyperplasia" involving several parathyroid glands was encountered. Following parathyroidectomy, the serum calcium levels returned to normal. The serum phosphorus values had previously returned to normal following pancreatic surgery.

The 2 cases not diagnosed until necropsy had bizarre clinical histories, which were not suggestive of altered endocrine function. In both instances, the pancreas and the adjacent duodenum were the sites of multiple islet cell tumors. In one, a metastasizing islet cell carcinoma was present also. The parathyroid glands in each patient showed "nodular hyperplasia"; evidence for function will be presented. In both cases, the adrenal glands were diffusely enlarged due to hyperplasia, and without evident function. In one case, an associated chromophobe adenoma of the pituitary gland was found.

THE TIME OF DEATH. Herbert P. Lyle and Frank P. Cleveland, Office of the Coroner and University of Cincinnati, Cincinnati, Ohio.

Pathologists engaged in the practice of forensic medicine often are requested to state the approximate time when death occurred either from violence or under unusual and suspicious circumstances. At the present time, there are several methods available for estimating the time of death but these methods are inadequate because the degree of error is so great. The present report is a summary of observations that have been made in the initial period of a 2-year research program. The specific aim is to determine the "normal" rate of post-mortem heat loss from the human body. The bodies are observed under environmental conditions that have been standardized by means of a constant temperature room. Each body is divested of clothing and is covered with a single cotton blanket. The temperatures of six different sites of the body are continuously recorded over a 24-hour period. Specially constructed thermo-

couples attached to a continuously recording machine measure the heat loss from the body. The temperature of each thermocouple is automatically recorded each minute during the period of observation. The data thus obtained are analyzed on the basis of Newton's law of cooling, whereby the rate of heat loss is dependent upon the differential temperature of the body and of the environment.

CIRRHOSIS OF THE LIVER IN WILD RATS. E. C. H. Schmidt* and Curt P. Richter, Memorial Hospital, Easton, Md., and Johns Hopkins Hospital, Baltimore, Md.

During a histologic survey of wild rats captured in Baltimore, Maryland, the authors observed a delicate, diffuse cirrhosis in the livers of most rats. The livers of 54 adults of varying sizes were studied in detail. Fifty-one showed some degree of scarring, which varied from dense, wide bands of fibrous tissue to a delicate, fibrillar, fibrous pattern coursing through the liver sections. Nineteen of the 51 livers which showed scarring had ova or worms of *Capillaria hepatica* in various stages of degeneration. Several causative factors were considered and explained in attempting to evaluate this condition. It was thought that it might be a characteristic lobulation similar to that seen in hogs, a toxic effect of certain food, a dietary deficiency such as is seen in choline deficiency, the result of bacterial or viral infection, the result of parasitic infestation, or a combination of two or more of these factors.

The livers of 8 additional wild rats weighing between 70 and 185 gm. were studied in detail in an attempt to identify the cause of this cirrhosis. Five of these rats, weighing less than 160 gm., had no cirrhosis and no parasites. The 3 rats weighing more than 160 gm. had worms and ova of *Capillaria hepatica* and granulomata arranged in the same diffuse parenchymal pattern as seen in the cirrhosis in the adult animals. The fact that the small rats did not have cirrhosis or inflammation in the livers and that the larger rats had worms and ova of *Capillaria hepatica* and an inflammatory reaction resembling the fibrous pattern seen in older rats led us to believe that *Capillaria hepatica* infestation was the cause of the cirrhosis described in this study.

THE INHIBITING EFFECT OF ESTROGENS ON THE DEVELOPMENT OF CIRRHOSIS AND HEPATOCELLULAR CARCINOMA IN RATS ON A CHOLINE DEFICIENT DIET. Paul Kotin,* Hugh A. Edmondson,* and Hans L. Falk, University of Southern California School of Medicine, Los Angeles, Calif.

Male rats were maintained on a modification of the György and Goldblatt choline deficient diet for the purpose of inducing hepatic cirrhosis. Along with the diet, natural and synthetic estrogenic substances were administered to various groups of test rats for the purpose of determining their effect on the development of cirrhosis and hepatocellular carcinoma. Control groups consisted of male rats receiving (1) the test diet without additional estrogen, (2) a stock laboratory diet with supplemental estrogens, and (3) a stock laboratory diet without additional estrogens. Female rats were divided into one test and three similar control groups for the assessment of any sexual differences in response.

The rats exclusively on the test diet showed early gross fatty changes in the liver followed by subacute fatty cirrhosis in from 90 to 120 days. Classical nodular cirrhosis with nodules measuring up to 6 mm. in diameter developed in these animals in 180 to 200 days. Histologic changes in the rats on the test diet alone were essentially similar to those seen in cirrhosis in clinical subjects. An additional remarkable feature was the accumulation of intracellular pigment primarily in the periportal areas. This pigment is presumably ceroid.

Other groups of male rats were placed on a regime of 200 $\mu$g. of estrogenic substances injected subcutaneously three times a week in addition to the test diet. Their livers at biopsy and necropsy were intensely congested. The capsules were smooth except for occasional 1 to 2 mm. areas of granularity. Irregular patchy areas of subcapsular hemorrhage up to 8 mm. in diameter were noted. The histologic find-

ings in this group consisted initially of changes associated with hypervolemia followed by, or simultaneous with, an unusual form of hepatocellular atrophy. Large lakes of blood developed that persisted until death. These lakes were initially intralobular, but in time they freely crossed periportal barriers. These lakes were noted to coincide with the subcapsular areas of blood accumulation seen grossly. Surrounding these lakes were narrow compressed cords of atrophic liver cells. Islands of distorted liver cells also could be seen in many of the blood pools. No specific sites of predilection for the initiation of these blood pools could be demonstrated. Fibrosis, if present at all, was minimal and primarily periportal. Hypervolemic changes could be demonstrated in other viscera in the male rats on estrogenic materials. Testicular atrophy was prominent. Female rats showed essentially the same qualitative hepatic response but the grade and intensity of change were significantly less.

Hepatocellular carcinoma did not develop in any of the test or control animals, in spite of marked cirrhosis in the test diet animals not receiving estrogens. The life span of the test diet rats as contrasted with that of stock diet rats was not significantly shortened.

Rats of both sexes on standard laboratory diet showed no hepatic changes. Certain similarities were noted between our experimental results and the natural history of clinical cirrhosis and the development of hepatocellular carcinoma.

THE PATHOGENESIS OF CARCINOMA OF THE LIVER. Paul E. Steiner,* University of Chicago, Chicago, Ill.

In 37 patients with primary hepatic carcinomas, about 40 per cent also had cirrhosis. The possible morphologic bridging forms in the cirrhotic livers included hepatocellular atrophy, hepatofibrosis, hepatic cell regeneration, bile duct proliferation, and cholangiolofibrosis. In contrast, no intermediate forms were seen in the non-cirrhotic livers, even small tumors appearing to spring *de novo*. Any concept of the pathogenesis of liver cancer must consider both situations.

It is possible that the abnormal cell forms in cirrhosis are causally unrelated to the histogenesis of carcinoma, and that cirrhosis is not precancerous but a lesion sometimes coexistent with cancer and caused by the same factors. The statistical association might further be explained by a heightening of susceptibility of the liver cells to carcinogenic factors by the hardship imposed by the cirrhosis. Thus the occurrence of cirrhosis alone and of cancer alone would be explained by the action of the same etiologic agents, and the combination present in about 5 per cent of cirrhotic livers would be of the nature of co-carcinogenesis.

PRIMARY CARCINOMA OF THE LIVER. Brooks H. Bishop, Joseph A. Cunningham,* and Walter F. Scott, Jr., Carraway Methodist Hospital and Medical College of Alabama, Birmingham, Ala.

This study is based on 5,030 necropsies (1946–1954) performed in four hospitals in Jefferson County, Alabama, which includes the city of Birmingham. According to the last census the population of this area is 59.1 per cent whites (predominantly of European origin) and 40.1 per cent Negroes (of African origin).

An analysis of this series yielded 30 cases of primary liver cell carcinoma and 166 cases of cirrhosis. When the series was broken down into sex-race groups, the highest incidence of liver carcinoma was found in Negro males (1.1 per cent). (This figure is interesting in that it is approximately the same as that reported by Berman for the African Bantus.) The incidence of cirrhosis is found to be higher in the white race, with the white male showing an incidence of 4.4 per cent; the white female, 3.8 per cent; the Negro male, 2.6 per cent; and the Negro female, 1.3 per cent. This finding was unexpected. It is at variance with reports from other parts of the world where a high incidence of primary liver carcinoma is usually associated with a high incidence of cirrhosis. It is noteworthy that, while only 31 cases of cirrhosis were found in 1,207 necropsies of colored males, in 14 of these (45.2 per cent) there was

also a primary carcinoma of the liver. In the white male group the incidence of primary carcinoma in association with cirrhosis was 7.4 per cent.

A study of these figures seems to indicate, at least for this area, that cirrhosis is less likely to develop in the Negro male than in the white male or female, but once cirrhosis develops in the colored male there is a much higher incidence of associated liver carcinoma.

THE HISTOCHEMISTRY OF ADENOSINETRIPHOSPHATASE AND 5-NUCLEOTIDASE ACTIVITY IN THE LIVER WITH BILIARY OBSTRUCTION AND CELLULAR NECROSIS. M. Wachstein* and Elizabeth Meisel, St. Catherine's Hospital, Brooklyn, N.Y.

In continuation of studies with substrates for specific phosphatases at a physiologic pH, techniques for the demonstration of adenosinetriphosphatase and 5-nucleotidase activity were applied to livers of rats and rabbits following experimental biliary obstruction and to liver obtained surgically for biopsy from patients with obstructive jaundice. In addition, the livers of rats with cellular necrosis induced by administration of carbon tetrachloride and with liver regeneration following partial hepatectomy were studied. Frozen sections prepared from tissue fixed in neutral chilled formalin gave, in general, better results than sections from unfixed tissue. The technique for adenosinetriphosphatase activity is excellent for the demonstration of bile canaliculi. A distinct and uniform staining of these structures is regularly observed even in species in which Gomori's stain for non-specific alkaline phosphatase gives either irregular (human) or negative (guinea-pig) results. In addition, with this technique activity is seen to a varying degree in the sinusoids as well as in structures located in periportal fields. 5-Nucleotidase activity using muscle adenylic acid as substrate, on the other hand, demonstrates almost selectively in the human liver the sinusoids and endothelium of blood vessels and less regularly the bile canaliculi.

Following the ligation of bile ducts, there occurs within 24 to 72 hours dilatation and tortuosity of bile canaliculi as well as marked accumulation of granules and rods in the vicinity of the bile canaliculi, which give a positive staining reaction. In later periods, however, bile capillaries show less enzymatic staining, particularly in the rat. In human livers, likewise, tortuosity of bile capillaries and accumulation of granular material in their vicinity was found. In several instances in which the obstruction has lasted for a longer time, many bile capillaries showed no enzymatic staining. Some appeared empty while others contained bile thrombi which gave a negative staining reaction. Increased tortuosity of bile capillaries was seen also in experimentally induced liver cell regeneration. On the other hand, cells undergoing necrobiosis fail to show any staining of the bile capillaries although occasionally sinusoids in their vicinity may demonstrate increased activity. The findings will be discussed in relation to their possible significance for the explanation of functional disturbances in biliary obstruction and liver cell necrosis.

THE PROGNOSTIC SIGNIFICANCE OF SO-CALLED MALLORY BODIES IN PORTAL CIRRHOSIS. John D. Rice, Jr., and Raymond Yesner,* Veterans Administration Hospital, West Haven, Conn.

Since their description by Mallory in 1911, alcoholic hyaline or Mallory bodies have often been described in livers showing a severe inflammatory phase of portal cirrhosis. Although not exclusively confined to portal cirrhosis, Mallory bodies are most often seen in that disease and have been regarded by many as indicating a poor prognosis when found in liver tissue obtained for biopsy. In an attempt to evaluate their significance, 307 liver biopsies in cases of portal cirrhosis or fatty metamorphosis of the liver from the files of the Newington, Conn., V.A. Hospital were reviewed. These covered the period from 1947 through 1954. From this material 20 cases showing unequivocal Mallory bodies in the usual hematoxylin and eosin stain were

compared histologically and with reference to clinical course with 20 cases showing advanced portal cirrhosis without evidence of Mallory bodies.

THE UPTAKE AND DISTRIBUTION WITHIN THE TISSUES OF LABELLED IRON ($Fe^{59}$) IN EXPERIMENTAL INFUSIONAL SIDEROSIS. Joseph Wack and John P. Wyatt,* St. Louis University School of Medicine, St. Louis, Mo.

The uptake by the globulin fraction of the blood, distribution, and ultimate reservoir of radioactive iron were studied in control rabbits and those with varying degrees of induced infusional siderosis. The localization of the radio-iron bound protein fraction was established by electrophoretic analysis. The search for "target" organs, as far as distribution of injected radioactive iron was concerned, was probed by scintillation methods and autoradiographic media. The explanation for these target organs in control and stimulated systems is interpreted in terms of reticulo-endothelial proliferation and conditioned pathways.

CIRRHOSIS OF THE LIVER IN SICKLE CELL DISEASE. Y. S. Song, University of Tennessee and City of Memphis Hospitals, Memphis, Tenn.

The lesions of the liver in sicklemia seem to be based on two mechanisms: (1) stagnation of the sickled erythrocytes in the hepatic sinusoids, with severe distention of the sinusoids producing central and paracentral cellular necrosis; (2) occlusion of the hepatic capillaries by agglutinative thrombi made up of the sickled red cells, resulting in anoxic necrosis of the cells.

The hepatic changes were studied in 31 post-mortem cases of sicklemia occurring in this institution in the past 20 years. Eighteen cases were in individuals under 20 years; the remaining 13 were 24 to 45 years of age. Gross examination revealed that the liver was consistently enlarged and deep purple or of mahogany color. The hepatic sinusoids were tremendously dilated and stuffed with sickled red blood cells, resulting in necrosis. Subsequent fibrous proliferation in the necrotic areas, destroying the normal architectural arrangement of the hepatic lobules, was observed frequently. Occasionally the sinusoids were practically occluded by hemolyzed material. Bile duct proliferation was rarely observed in the scar tissue.

The degree of hemosiderosis seemed to be variable and not related to the duration of the disease. Most of the hemosiderin was seen in the hepatic cells and only a small amount was present in the Kupffer cells. Generally the Kupffer cells were slightly swollen, but otherwise not remarkable. The hepatic capillaries were occluded by agglutinative thrombi composed of the sickled cells in 19 per cent of the patients. Cholelithiasis and chronic cholecystitis were observed in 2 cases. The intrahepatic and extrahepatic bile ducts and gallbladder of the remaining cases were not unusual. Cirrhosis was observed in 9 patients ranging in age from 14 to 35 years. In these cases the liver contained multiple nodules of varying sizes separated by broad fibrous tissue. The gross appearance of the liver certainly was suggestive of the type of post-necrotic cirrhosis.

From the above data it seems apparent that the prolonged sinusoidal stagnation and gradual vascular occlusion by the sickled erythrocytes result in cellular necrosis and that the necrotic cells are replaced by fibrous tissue eventually terminating in progressive, irreversible cirrhosis.

SECONDARY HEMOCHROMATOSIS IN BLOOD DYSCRASIAS, WITH SPECIAL EMPHASIS ON ERYTHREMIC MYELOSIS. Geoffrey Kent, Harold Schrifter, and Hans Popper,* Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill.

In blood dyscrasias associated with anemia a hemochromatosis may develop which differs from hemosiderosis in the distribution of the iron pigment and from primary hemochromatosis in the rapidity of the process. In secondary hemochromatosis the hepatic connective tissue septa are delicate and consist mainly of newly formed reticulum rather than collagenous fibers. The rapidity of the process is

brought out well in a case of refractory anemia due to Hodgkin's disease by a marked difference in iron content of the liver between a biopsy specimen taken 3 months before death and the necropsy specimen. The florid type of hemochromatosis is explained by alteration of iron metabolism induced by the anemia and aggravated, but not caused, by iron medication and transfusions. The fact that not all chronic anemias are associated with hemochromatosis indicates the presence of additional factors. The incidence of hemochromatosis appears strikingly high in proliferative disorders of the erythropoietic series and 3 such cases are reported.

The cases are those of a 75-year-old women and of 70- and 65-year-old men. Symptoms of anemia extended over 19 to 35 months. The clinical hematologic diagnoses were erythroleukemia in one case, erythremic myelosis in the second case, and "uncertain" in the third case. Necropsy revealed hemochromatosis in all 3 cases, together with proliferation of reticulum cells and the early red cell series. All cases had profound normoblastic or megaloblastoid hyperplasia of the bone marrow, with maturation arrest, absence of hemolytic anemia by routine procedures, infiltration of the organs with early erythropoietic elements, and lack of response to known hematinics. In one case, the presence of primitive white cells suggested the diagnosis of erythroleukemia, while the other 2 cases were considered examples of erythremic myelosis.

The association of proliferation of the erythropoietic series with hemochromatosis suggests that such proliferative processes, possibly neoplastic in nature, may represent one factor in the alteration of the cellular iron metabolism leading to increased cellular avidity for iron.

THE OCCURRENCE OF GLYCOGEN IN SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX.† Alvan G. Foraker,* Baptist Memorial Hospital, Jacksonville, Fla.

Absence of glycogen in squamous cell carcinoma of the cervix is almost axiomatic in gynecologic pathology, and has been proposed as a practical diagnostic point in evaluating tissue sections and exfoliated cells. In previous studies, we found glycogen reactions in well differentiated squamous cell carcinoma of non-cervical origin. In the present investigation, specimens for biopsy from 21 examples of invasive squamous cervical carcinoma were fixed in alcohol and then subjected to the periodic acid-Schiff technique for the demonstration of glycogen. Control sections were digested with malt diastase. Thirteen of the 21 cases showed some degree of glycogen staining of the cytoplasm of some of the carcinoma cells. This was usually present in "maturing" cells in the centers of neoplastic masses. Perhaps some of the discrepancies in the literature are due to differences in fixation and staining techniques, such as applying to glycopenic carcinoma cells procedures adequate for glycogen demonstration in glycogen rich tissues such as rat liver. Caution is advised in interpreting absence or presence of glycogen staining in smears or sections as a valid criterion in the diagnosis of squamous cell carcinoma of the uterine cervix.

IDENTIFICATION OF ALPHA-AMYLASE AS A SECRETION FROM THE HUMAN FALLOPIAN TUBE AND EPITHELIUM OF MÜLLERIAN AND MESONEPHRIC DUCT ORIGIN. Charles L. Green, University of Texas Southwestern Medical School and St. Paul's Hospital, Dallas, Texas.

Fluid from 72 cystic structures from the female pelvis lined by epithelium morphologically resembling that of fallopian tube epithelium was quantitatively assayed for diastase enzyme activity. Somogyi's micro-method for determining diastase activity was used.

The structures were divided into four groups composed of: (1) Twenty-eight

† Supported by Research Grants C–2111 and C–2392 from the National Cancer Institute, National Institutes of Health, Public Health Service.

cysts of mesonephric tubule and duct origin, including epoophoron, paroophoron, and parovarian cysts. The average diastase activity was 8,600 units. (2) Eight cysticly dilated fallopian tubes showing no evidence of active inflammation. The average diastase activity was 4,300 units. (3) Thirty-six cysts of müllerian duct origin, including hydatids of Morgagni and müllerian cysts of the tube wall. The average diastase activity was 17,000 units. (4) Two hydatids of the testes of müllerian duct origin and lined by "tube-like" epithelium. Diastase activity was 60,000 and 80,000 units respectively.

Fluids from a number of cystic structures from other embryologic origins were assayed and showed no diastase activity.

The following chemical and physical properties of this enzyme led to the conclusion that it is most likely an alpha-amylase. (a) It splits alpha 1-4 glucocidic bonds in polysaccharides. (b) It will not liberate phosphate or synthesize glycogen from glucose-1-phosphate. (c) It becomes inactive when dialyzed against distilled water and is reactivated by the addition of chloride ions. It was shown that this enzyme can degrade glycogen produced by secretory endometrium to glucose. In the human only extracellular glycogen is degraded by alpha-amylase while intracellular glycogen, under physiologic conditions, is metabolized by phosphoralases.

From these findings the hypothesis is advanced that alpha-amylase is a physiologic secretion of the epithelium of the fallopian tube. The probable substrate for this enzyme is the intraluminal glycogen derived from secretory endometrial glands. High implantation of the ovum would be favored because of available glucose for nutrition near the tubal ostia during the secretory phase of the endometrial cycle.

THE RELEASE OF ENTEROCHROMAFFIN CELL SUBSTANCE BY RESERPINE.† Earl P. Benditt* and Ruth L. Wong, University of Chicago and LaRabida Jackson Park Sanitarium, Chicago, Ill.

Reserpine has been found to reduce the content of serotonin (5-hydroxytryptamine) in the intestinal tract of rabbits. Current evidence indicates that the material giving the characteristic enterochromaffin reactions is a hydroxyindole, probably serotonin. Examination of the enterochromaffin cells of rabbit stomachs and guinea-pig duodenums 16 hours after the administration (5 mg./kg. body wt.) of reserpine showed a substantial decease (80 per cent) in the number of visible argentaffin cells. This decrease fits well with the values reported for the decrease in serotonin concentration of rabbit intestinal tract following this dose of reserpine. The evidence, therefore, is consistent and indicates that at least one site of action of reserpine is the enterochromaffin cells. The same dose of reserpine given to rats produces acute gastric hemorrhage and superficial ulceration of the mucosa in the glandular portion of the stomach. Enterochromaffin cells are not discernible in the rat stomach by the usual techniques and as yet no clearly defined cellular lesion has been discerned.

These observations are consistent with the notion that reserpine may act, at least in part, by stimulating the release of physiologically active material, such as serotonin.

STUDIES ON THE CORRELATION BETWEEN MORPHOLOGIC CHANGE AND ALDOLASE ACTIVITY IN NEUROMUSCULAR DISEASES. Bruno W. Volk* and Stanley M. Aronson,* Jewish Chronic Disease Hospital and State University of New York, College of Medicine, Brooklyn, N.Y.

The serum aldolase level was studied in 233 patients with a wide variety of neuromuscular disorders. In many patients muscle biopsy specimens were obtained also. In 33 cases of progressive muscular dystrophy the serum aldolase concentra-

† Supported in part by grants (H-2141) from the United States Public Health Service, the American and Chicago Heart Associations.

tion invariably was elevated. The magnitude of this increase was most pronounced in young children, the degree of elevation diminishing progressively with increasing age. Monthly or bi-monthly serial aldolase determinations, carried out on several individuals with muscular dystrophy up to one year, disclosed a gradual decline of the initially elevated serum enzyme level. In all instances in which muscle tissue was studied, elevated *serum* aldolase values were inversely proportional to a markedly decreased *tissue* enzyme activity. In a limited number of these patients, parallel determinations of tissue phosphorylase also showed a marked decrease of this enzyme in the excised muscle as compared with normal values. The histologic alterations characteristic of muscular dystrophy were considered the fundamental criterion of diagnosis, although protracted clinical evaluation also was taken into account. The extensiveness of the dystrophic process, as judged by clinical progression and histologic appearance, correlated well with the parallel aldolase levels. The data seemed to indicate that the sustained serum elevation of this enzyme is the result of enzyme release from continuing striated muscle degeneration.

In nuclear myelopathies exemplified by progressive degeneration of the spinal anterior horn cells (e.g., amyotonia congenita, arthrogryposis multiplex congenita, Tay-Sachs' disease, amyotrophic lateral sclerosis), a moderate hyperaldolasemia was apparent. Patients in the category of upper motor neuron lesions (vascular hemiplegia), diffuse cerebral degenerative disease (Schilder's disease, multiple sclerosis), or wasting infirmity of non-neurologic origin (e.g., incapacitating arthritis), invariably displayed normal or subnormal serum aldolase values in spite of evident muscular atrophy.

To explain some of these results, dogs were submitted to femoral neurotomy or tenotomy of the Achilles tendon. Serial determinations disclosed a slightly belated but significant rise in serum aldolase following neurotomy, while muscular atrophy induced by the tenotomy, with preservation of the nerve supply, was not attended by any rise in the serum aldolase. Serial biopsy samples from the involved extremity showed a gradually diminishing diameter of the skeletal muscle fibers, regardless of the type of induced atrophy.

The data of this study suggest that significant elevation of serum aldolase occurs particularly in young patients with progressive muscular dystrophy. The experiments, which support, in part, the clinical experiences, seem to indicate that a significant but moderate elevation of this enzyme in serum occurs in those forms of muscular atrophy consequent to functional severance of the lower motor neuron complex. In contrast, muscular atrophy of non-neurologic origin or due to diffuse cerebral or upper motor neuron disorders is unattended by serum aldolase elevation.

MYASTHENIA GRAVIS. II. THE HISTOCHEMICAL DEMONSTRATION OF ACETYLCHOLINESTERASE IN THE MOTOR END-PLATES OF EXTRA-OCULAR MUSCLE IN MYASTHENIC AND NON-MYASTHENIC SUBJECTS. R. B. Cohen, S. Zacks, and B. Castleman,* Massachusetts General Hospital, Boston, Mass.

Extra-ocular muscle was obtained several hours after death in a series of 5 patients who died as a result of myasthenia gravis. All patients had large doses of prostigmin immediately prior to death. The motor end-plates of these muscles were studied utilizing a histochemical technique for the demonstration of cholinesterase, and were compared with a series of control muscles obtained from non-myasthenic patients. In only one instance did the acetylcholinesterase activity of the myasthenic end-plate appear decreased. The significance of these results and the value of extra-ocular muscle as a source of material for the study of motor end-plates are discussed.

## Symposium on Virus Disease

Referee (by invitation of the Council): Albert B. Sabin

Immunogenic or Protective Factor in Fecal Contents of Mice Infected with Mouse Encephalomyelitis Virus. Alvar A. Werder and Thorkil Jensen, University of Kansas School of Medicine, Kansas City, Kans.

Three-weeks old CFW mice were injected with the GDVII strain of Theiler's mouse encephalomyelitis virus by three routes, viz., oral, intravenous, and intracerebral. Feces, blood, and brains were collected over a 21-day period from these mice and injected intracerebrally into other mice. Twenty-six per cent of these animals developed paralysis and died. The survivors then were challenged with 100 $LD_{50}$ intracerebral doses of GDVII virus. The fecal contents from the orally injected mice protected 53 per cent of the mice against the subsequent intracerebral challenge by injection of GDVII virus; whereas fecal contents from non-inoculated mice protected only 18 per cent of the recipients of such a challenge injection. Blood from the orally inoculated mice conferred protection in 22 per cent of the mice. None of the other specimens protected intracerebrally injected recipients. In these studies over 1,000 mice were used.

The nature of the immunogenic component responsible for this protection was investigated by experimental procedures which were designed to check these possibilities: (1) viral inhibitor, (2) dead virus, (3) subclinical dose of virus, and (4) avirulent variant. The results of these studies suggest that the concept of an avirulent variant offers a likely explanation for this protection.

Loss of Cytotoxicity of Newcastle Disease Virus for Cells in Tissue Culture. Earl J. Mason and Nathan Kaufman,* Cleveland City Hospital, Cleveland, Ohio.

Massive inocula of Newcastle disease virus (NDV) were found to be cytopathogenic for three cell strains maintained *in vitro*. These were strain L and strain LLCM1 of murine origin and strain $U_{12}$ from human uterine tissue. The toxic effect of the NDV-infected allantoic fluid became greater as the ratio of egg-infectious virus particles in the inoculum to cells increased. No definite evidence for viral multiplication could be obtained in cultures of any of the three cell strains.

It was found that cytopathogenicity of the virus could be altered by serial alternate tissue culture and egg passage. Cultures of strain L cells were inoculated with infected allantoic fluid and samples of supernatant were titrated at intervals. At a time when most egg-infectious virus had disappeared, a duplicate sample was used to inoculate fertile eggs, in order to harvest progeny of the surviving viruses. The infected allantoic fluid was then used to inoculate a new set of L cell cultures and the cycle was serially repeated. After these procedures the virus (in allantoic fluid) had retained its egg-infectivity but had lost its cytopathogenicity; furthermore, there was no demonstrable toxicity when massive inocula of the altered virus were placed in cultures of LLCM1 and $U_{12}$ cells. This alteration was not specific toward the cell strain in which it had developed. The loss of toxicity without concomitant loss of egg-infectivity would indicate that these two manifestations depend on separate viral moieties.

Stable and Pure Line Human Cells in Continuous Culture for Studies of Polioviruses. J. T. Syverton* and LeRoy C. McLaren, University of Minnesota School of Medicine, Minneapolis, Minn.

A variety of human normal and malignant cells have been employed for studies *in vitro* of viruses by use of cell cultures derived from explants by trypsinization or

other means of dispersion, or of stable cell strains in continuous culture. A refinement in cell culture is the use of a pure line of cells derived from a single cell. This approach has been widened by the method of Puck, Marcus, and Cieciura for establishing clonal growth of mammalian cells *in vitro*. The application of a modification of the Puck technique has resulted in this laboratory in multiple pure lines of normal and malignant cells for comparative virus studies. The present report is limited to studies of the comparative response to types 1, 2, and 3 poliovirus infection of monkey-kidney cells for control purposes, stable cancer cell strains HeLa and Maben, stable normal cell strains derived from human liver and conjunctiva, and a pure strain of human liver epithelial cells. The pure strain of human liver epithelial cells multiplies more rapidly than the parent stable liver cell line, and comparative titrations indicate that the pure cell strain is equally as susceptible to infection by types 1, 2, and 3 poliovirus as the other cell culture systems.

PROPAGATION OF A CYTOPATHOGENIC VIRUS FROM SALIVARY GLAND VIRUS DISEASE OF INFANTS IN TISSUE CULTURES. Margaret G. Smith,* Washington University School of Medicine, St. Louis, Mo.

Greatly enlarged cells containing large intranuclear inclusions are observed in the salivary glands of some infants, regardless of the cause of death, and are present in other organs, as well as the salivary glands, in cases of generalized cytomegalic inclusion disease. These characteristic inclusions resemble closely those occurring in the salivary glands of rodents, and in rodents the inclusions have been shown to be caused by species-specific filtrable viruses, designated as the salivary gland viruses. It is generally accepted that the human disease, whether latent in the salivary glands or generalized, is caused by an analogous filtrable virus. However, isolation and propagation of a virus from the human disease has not been reported previously.

Roller tube cultures of fibroblasts (spindle cells) derived from human myometrium were maintained at 34° to 35° C. in a nutrient medium consisting of Hanks's balanced salt solution and ox serum ultrafiltrate (2:1), chick embryonic extract (10 per cent) and horse serum (10 per cent). Penicillin and streptomycin were added. Two cytopathogenic agents, producing morphologically identical cellular changes, have been propagated serially in the cultures of human tissue. The first strain was isolated from a submaxillary salivary gland of a 7-months-old infant whose death was caused by adrenal cortical carcinoma. Histologic examination of the other submaxillary gland revealed many intranuclear inclusions characteristic of salivary gland virus disease. There was no visceral involvement. The second strain was isolated from the kidney of a 1-month-old infant who had generalized salivary gland virus disease (cytomegalic inclusion disease). The first strain has been maintained for 25 serial passages over a period of 2½ years; the second, for 8 serial passages over a period of 10 months.

Cytopathologic changes developed slowly in the first isolations and in the earlier subcultures of each strain. They were observed first in a few isolated groups of cells from 1 to 4 weeks after inoculation of cultures. The affected cells were enlarged, rounded, and slightly refractile in contrast to surrounding spindle cells. The size and number of the focal lesions slowly increased and degeneration of cells in larger lesions occurred. Even after 2 months many cells of the cultures were not altered. The first three serial subcultures of each strain of virus were successful only when preceding cultures had been maintained for at least 1 month and when the entire 2 ml. of undiluted fluid medium from preceding infected cultures was transferred to the new cultures. In successive serial passages cytopathologic changes appeared earlier and spread more rapidly to involve the entire culture. At present, passages are made by transferring 0.2 ml. of fluid from infected cultures to each new culture. Cytopathologic changes appear in the cultures in 2 to 5 days when the successive serial passages are made at intervals varying from 3 weeks to 9 days.

In stained, fixed cultures large intranuclear inclusions comparable to those seen in human salivary gland virus disease are present in the greatly enlarged cells. The intranuclear inclusions are morphologically identical to those produced by the mouse salivary gland virus in cultures of mouse tissue. The agents from human salivary gland virus disease could not be serially propagated in cultures of mouse tissue, nor were they infective for the mouse by intracerebral inoculation. Also, they were not infective for the embryonated hen's egg when inoculated on the chorioallantoic membrane.

ELECTRON MICROSCOPY OF SALIVARY GLAND VIRUSES.† Sarah A. Luse and Margaret G. Smith,* Washington University School of Medicine, St. Louis, Mo.

Fibroblasts derived from human myometrium were grown in tissue culture and inoculated with salivary gland virus. The virus was obtained at necropsy from the submaxillary gland of an infant. The contralateral gland contained intracellular inclusions characteristic of salivary gland virus infection. At intervals following inoculation, cells from tissue cultures were fixed in 1 per cent bichromate-buffered osmic acid, dehydrated, and embedded in methacrylate. Thin sections were examined in an EMU-2E RCA electron microscope while adjacent thicker sections of the same cells were examined by phase microscopy. By the latter method, the diseased cell was characterized by an enlarged nucleus containing a central inclusion surrounded by a clear halo. Intracytoplasmic and intranuclear particles, probably viral, were invariably identified in electron micrographs of diseased cells, whereas none was noted in control uninfected cultures. The particles contained a central dense dot of osmiophilic material, surrounded by a zone of lighter substance which was in turn enclosed by an outer osmiophilic shell of varying thickness. Transitions were seen from these structures to ones in which the central body was enlarged so as to fuse with the outer shell. The nuclear inclusions were composed of these particles interspersed among irregularly clumped, finely granular, chromatin material.

The cytoplasm of the infected cells contained particles identical with those described and, in addition, larger spherical bodies of dense homogeneous composition. Both types of cytoplasmic particles were enclosed within vacuolar structures set off from the surrounding cytoplasm by an osmiophilic membrane. These vacuoles contained sometimes one particle and sometimes many. The vacuolar wall was similar in appearance to the membrane bounding the Golgi tubules which were prominent in the infected cells.

Infected cells from the spleens of young mice following intraperitoneal inoculation with the mouse salivary gland virus were examined by phase and electron microscopy. These cells contained particles similar to those in the tissue culture cells, whereas, in the splenic cells of control animals, particles were absent.

AN INCLUSION-BODY-PRODUCING VIRUS RECOVERED FROM SPONTANEOUSLY DEGENERATING TISSUE CULTURES OF HUMAN ADENOIDS. Wallace P. Rowe, Janet W. Hartley, and Robert T. Huebner, National Institutes of Health, Bethesda, Md.

During surveys for the presence of APC viruses in tissue cultures of adenoids of children undergoing tonsillectomy-adenoidectomy, three strains of a cytopathogenic virus were recovered from cultures in which the fibroblasts underwent spontaneous degeneration. The virus produces cytopathogenic effects only in fibroblasts, inducing focal areas of necrosis and pigmentation, with many type A intranuclear inclusion bodies. Culturally and serologically the virus appeared closely related or identical to the agents previously recovered by Smith from human salivary gland tissue and

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by Weller from a case of cytomegalic inclusion disease. Complement fixing and neutralizing antibodies are widely prevalent in the general population, the incidence increasing progressively with age from about 20 per cent in infants to 80 per cent in adults. Present evidence suggests that these agents represent the virus responsible for human salivary gland inclusions and cytomegalic inclusion disease.

INCLUSION BODY (VIRAL) DISEASE IN THE NEWBORN. Tobias Weinberg* and Alma B. Kelly, Sinai Hospital, Baltimore, Md.

Four cases are presented of infants, 3 of whom died within the first few weeks of life and one, stillborn, in whom intranuclear inclusions were found in either the adrenal glands or liver, or both. One case showed a histologic picture of liver, adrenal, splenic, gastric, and esophageal necrosis as described by Zuelzer in the cases proved by him to be typical of herpes simplex virus septicemia. Another case showed involvement of the liver in a fashion which others have interpreted as characteristic of cytomegalic inclusion disease. In the stillborn infant, intranuclear inclusions were found only in the adrenal glands. The differentiation of herpes simplex virus septicemia from cytomegalic inclusion disease does not appear to be clearly defined. It is suggested that the 4 cases reported present a variation on a theme, with severity and extensiveness of involvement deciding the clinical picture and its part in the death of the infant. Whether cytomegaly is sufficient to indict separate viruses is a moot point.

EFFECT OF CORTISONE ON THE GROWTH OF VACCINIA VIRUS IN TISSUE CULTURE. Margaret Holden and Lillian B. Adams, College of Physicians and Surgeons, Columbia University, New York, N.Y.

Hydrocortisone (free alcohol, 50 μg./ml.) incorporated in culture media of actively proliferating mouse fibroblasts (L strain, Earle) induces the development of abnormal morphologic changes and delays the fall in pH of the medium.

The effect of cortisone on the capacity of fibroblasts to support growth of vaccinia virus was studied. Culture tubes of fibroblasts, one set pre-treated with cortisone for 10 days, were inoculated with serial ten-fold dilutions of virus-infected mouse brain. Distinct foci of degeneration occurred in both untreated and cortisone-treated cells within 2 to 7 days after the addition of the virus. In the untreated series of cultures, the cells became rounded and granular, and showed a tendency to clump, forming plaques surrounded by many healthy fibroblastic cells. In contrast, the virus caused more destructive effects in the cortisone-treated cells, which became swollen and confluent with indistinct cell membranes; the plaques were smaller and more numerous, and there were many areas of degeneration. This was probably due to enhanced growth of virus in the cortisone-treated cultures, since the infectivity titers of culture supernatants were 1 to 3 log units higher than those from cultures of untreated cells. The characteristic cytopathogenic effect of vaccinia virus in both normal and cortisone-treated cells has persisted through 15 passages over a period of 6 months. This effect can be specifically inhibited by immune serum.

Titration in mice and rabbits from selected passages provided further evidence that the virus multiplied to a greater extent in cortisone-treated cells. Mice were inoculated intracerebrally with successive ten-fold dilutions of virus from untreated and cortisone-treated cultures. In all instances, death occurred more rapidly and the $LD_{50}$ was consistently 1 to 3 log units higher in the animals which received material from cortisone-treated cultures. Skin lesions in rabbits following injection of dilutions of virus from cortisone-treated cultures showed greater induration and necrosis when compared with lesions caused by similar dilutions of virus from normal cell cultures, and the infectivity end-points were 1 to 3 log units higher.

INCREASED VIRUS POTENCIES IN CULTURES TREATED WITH HYALURONIDASE (ALIDASE).† Murray Sanders* and M. G. Soret, University of Miami, Coral Gables, Fla.

Hyaluronidase has been used successfully as a tissue reactor in cultures infected with Eastern equine encephalomyelitis or poliomyelitis viruses. A commercial grade of hyaluronidase, Alidase, has been used because of its availability and reliability.

The EEE virus was studied in modified Maitland chick embryo tissue cultures, and potency of the agent was determined by intracerebral inoculation of CFW mice. On the basis of mouse tests the infected cultures which contained Alidase yielded from 10 to 30 times more virus than the control cultures. Titers as high as $10^{-7.75}$ were obtained from the Alidase cultures. This constitutes the most potent yield reported to date for an animal virus in tissue culture, where the potency test was based on mouse infectivity. The EEE studies were carried out at 37° C. and at room temperatures varying from +5° to 20° C. At 37° C. as much as one million-fold increase in the virus was noted in the first 24 hours. At room temperatures the growth rates were generally slower before reaching the maximum potency of $10^{-7}$. The Alidase potentiating action was also apparent at room temperatures and to the same degree as that noted at 37° C.

Mahoney and MEF I strains of type I and type II poliovirus, respectively, were included in the Alidase study. Virus potencies were determined by cytopathic effect in monkey kidney and HeLa tissue cultures. As expected, the virus titers for both strains of poliovirus were higher in the monkey kidney cell cultures than in HeLa cultures. In both types of tissue cultures the presence of Alidase effected an increase in potency of the order of 10 to 30 times compared to control cultures.

The rôle of Alidase in modified Maitland cultures containing the virus of St. Louis encephalitis, and marine embryonic cultures infected with EEE virus, is under study and will be discussed.

It is suggested that in view of the small amounts of Alidase required to potentiate virus propagation and because of the known action of hyaluronidase, the type of culture here reported should be useful in primary virus isolation and in vaccine production. The Alidase technique appears to be an improvement over Cortone as a virus culture potentiator.

VIRAL ETIOLOGY OF MOUSE LEUKEMIA. Ludwik Gross, Veterans Administration Hospital, Bronx, N.Y.

Recent experiments have demonstrated that leukemia in mice is caused by a filterable, thermolabile agent, transmitted from one generation to another directly through the embryos. In most instances, this agent, though present in the carrier-host since birth, causes no symptoms of disease. In some instances, however, usually in middle aged hosts, and for obscure reasons, the hitherto latent agent may change into a formidable pathogen, causing then symptoms of leukemia and thereby sealing the fate of its carrier-hosts. The survival of the agent, however, has been as a rule assured, the agent having been passed to the next generation of hosts prior to the development of disease in the carrier. Frequently, the carrier may remain in perfect health, even though it transmits the leukemic agent to its offspring. Thus, leukemia may skip one, or several successive generations of carriers. This epidemiologic pattern may be responsible for the great difficulty in tracing the course of natural transmission of the leukemic agent, unless several successive generations of a given family of carrier-hosts are observed and recorded through their natural life-span.

The presence of the submicroscopic leukemic agent can be determined in mice by bioassay. This consists of inoculating newborn mice of a susceptible strain, free from spontaneous leukemia. Following such inoculation, some of the injected mice develop leukemia after an interval varying from a few months to 1 or 2 years.

† Produced by G. D. Searle & Co., Chicago, Illinois.

The difficulty in understanding the nature of leukemia in mice is increased by the observation that cell-free (centrifuged or filtered) leukemic extracts, injected into susceptible mice, may cause, instead of leukemia, other types of malignant tumors, such as salivary gland carcinomas or soft tissue sarcomas. Whether one agent may be able to cause different types of new growth, or whether the leukemic extracts prepared from leukemic mice and used for inoculation contain a mixture of distinct oncogenic agents, remains to be determined.

THE MORPHOLOGY OF CELL-FREE-FILTRATE-INDUCED LYMPHOMA IN THE AKR MOUSE. Paul B. Szanto,* Harold M. Schoolman, and Steven O. Schwartz, Hektoen Institute for Medical Research of the Cook County Hospital, Chicago, Ill.

When 4- to 16-week-old AKR mice are inoculated intracerebrally or intraperitoneally with cell-free filtrates of lymphomatous AKR mouse brain, lymphoblastoma develops in more than half of the animals within 3 to 5 weeks. Spontaneous lymphoblastoma occurs with a frequency of less than 1 per cent in our substrain at this age. The filtrate is bacteriologically sterile, will pass a Seitz filter, withstands prolonged freezing, and is heat labile. Control experiments which have included filtrates, prepared in an identical fashion from the tumor tissue of the same animals whose brains are used, yield negative results with the exception of an occasional animal which developed leukemia when inoculated with cell-free filtrate of human leukemic brain.

Gross pathologic changes are enlargement of the thymus, liver, and spleen, and a generalized lymphadenopathy. Microscopically, the type of involvement is constant, but considerable variation occurs in the degree of the involvement of the various organs. The affected lymph nodes show a complete or partial replacement by tumor cells which invade the capsule. These cells are uniform in type, have relatively large spherical nuclei and little basophilic cytoplasm. This is classified as lymphoblastic lymphosarcoma. Less frequently, the tumor cells show much greater pleomorphism, being made up of cells with ovoid or indented nuclei and large nucleoli. Sections impregnated with silver fail to reveal argentophilic fibers. Large sheets of tumor cells replace the hepatic parenchyma. The nuclei of the hepatic cells are frequently enlarged. The spleen is less frequently involved. Megakaryocytes, which normally occur in the mouse spleen, are usually preserved. The thymus is usually replaced by tumor tissue. Animals inoculated intracerebrally show infiltration of the meninges by tumor cells which extend into the Virchow-Robin spaces. The kidneys, pancreas, lungs, marrow, and intestine show varying degrees of involvement.

While the nature of the filterable agent has not yet been clarified, two observations are particularly pertinent. First, the "incubation" period is of the order of magnitude of whole tissue transplants and remarkably shorter than is to be anticipated with so-called leukemogenic agents. In this connection it is to be noted that a single inoculation of a greatly diluted, small dose is used. Secondly, unlike other known leukemogenic factors, this agent is derived from brains of lymphoblastomatous animals.

CYTOPATHOGENIC EFFECTS OF VIRUSES ON HUMAN TUMORS. Alice E. Moore, Sloan-Kettering Institute, Memorial Center, New York, N.Y.

A number of cell lines derived from human tumors have been studied to determine the cytopathogenic effects of neurotropic viruses. Growth curves have been determined by exposing cells to the virus, washing off the excess after adsorption has taken place, and doing daily titrations thereafter. It was found that, in general, the shape of the growth curve for a given virus was the same in whatever cell line it multiplied, and whether the virus exerted a cytopathogenic effect or not. In some instances it was possible also to study the effects of the same viruses on human tumors grown on the chorioallantoic membrane of the egg and subcutaneously in irradiated

and cortisone-treated rats. It was found that a virus which was effective against a tumor grown in tissue culture was also effective against those grown in the other situations, although usually not to the same degree.

CORRELATED HISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDIES OF INTRANUCLEAR CRYSTALLINE AGGREGATES OF RI-APC VIRUS IN HeLa CELLS. D. P. Bloch, C. Morgan, G. C. Godman, C. Howe, H. M. Rose, and D. H. Moore, College of Physicians and Surgeons, Columbia University, New York, N.Y.

Electron microscopy of thin sections of HeLa cells infected with RI-APC virus has revealed spherical viral particles approximating 60 $\mu\mu$ in diameter. Intranuclear crystals composed of virus were repeatedly encountered. The crystals, averaging 2 to 3 $\mu$ in diameter, often exhibited characteristic flat, angulated faces. Many nuclei also contained diffusely scattered virus or aggregates of particles oriented at random. A hypothesis has been presented elsewhere concerning the sequential stages in the development, crystallization, and release of the virus.

To characterize the crystals and aggregates histochemically, alternate thin (0.04 $\mu$) and thick (2 to 3 $\mu$) serial sections were cut permitting examination of contiguous sections of the same cell with both the electron and light microscope. The infected cells were prepared by centrifugation, fixation in buffered osmium tetroxide, dehydration, and imbedding in methacrylate. The aggregates and crystals were found to be Feulgen positive, demonstrating the presence of DNA. Because the electron microscope reveals the crystals to consist of a close-packed array of viral particles without identifiable interstitial material, their Feulgen color would indicate that the DNA is in the virus. On application of azure B after the Feulgen reaction, some of the intranuclear material including the chromatin, previously seen to be Feulgen positive, is found to be basophilic, but most of the large viral aggregates hardly stain with azure. This suggests that their DNA is differently combined with protein. Also in contrast to the chromatin, the intranuclear aggregations fail to stain with the alkaline fast green method for histone, presumably showing that they lack basic protein of this type.

PATHOGENESIS OF POLIOMYELITIS. REAPPRAISAL IN THE LIGHT OF NEW DATA. Albert B. Sabin (Referee†), Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, Ohio.

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OBSERVATIONS ON THE ORGANIZATION, DEVELOPMENT, AND ULTRAMICROSCOPIC STRUCTURE OF THE CAPILLARIES OF THE RENAL GLOMERULUS. Vincent Hall, University of Illinois, Urbana, Ill., and Argonne National Laboratory, Lemont, Ill.

Electron micrographs and photomicrographs demonstrating the "filtration apparatus" ("basement membrane complex") of the glomerular capillaries of the rat, rabbit, and man will be shown. Evidence will be presented showing that the glomerular capillaries are organized into functional and anatomical units, the lobules, within which are 2 or 3 relatively large vessels serving as direct channels (perhaps functioning as by-passes for erythrocytes) from the afferent to the efferent end of the lobule. These large capillaries give rise to numerous smaller and often very short branches which join and divide freely to constitute a true capillary network, forming a complex, unified system connecting laterally into the direct communicating channels which form the limits of the lobule. This description of the organization of glomerular capillaries is fully supported by detailed photographs of latex injected glomeruli, by analysis of a photographic reconstruction of a glomerulus from 2 $\mu$ serial sections, and by the evidence of electron micrographs.

† By special invitation of the Council.

It is often impossible to identify the numerous, short, anastomosing capillaries within each lobule in a single histologic section of a glomerulus. Their reality is readily seen in sections only when thin (1 to 3 $\mu$) sections are followed serially. When such techniques are employed, cells, which in a single section may appear as mesangial, in a section 1 to 2 $\mu$ away appear unquestionably as endothelial. The electron microscope and thin serial sections reveal only two cell types associated with the glomerular capillaries: endothelial cells forming the capillary walls, and so-called epithelial cells covering them.

The electron microscope reveals that the glomerular capillaries are completely lined, except in regions around the endothelial nuclei, with a specialized, thin (300 to 500 Å) layer of cortical endothelial cytoplasm (lamina attenuata), which, in perpendicular and tangential sections, frequently appears to be perforated by numerous pores about 400 to 900 Å in diameter. In areas these pores have a maximum frequency of about $5 \times 10^9$ per cm.$^2$ The external surface of the attenuated endothelium, as well as the thicker regions of all endothelial cells, is in intimate contact with the uniform, dense, definitive basement membrane (lamina densa) measuring from 600 Å to two or three times that in thickness. Apparently this is the only portion of the basement membrane complex which could possibly be sufficiently finely porous to serve as an ultrafilter, but, as yet, the fine porous nature of the lamina densa has not been visualized with certainty. The external surface of the lamina densa is covered intimately with a layer, about 2,000 Å thick, of interdigitating minute terminal pedicels (foot processes), derived from and in continuity with the overlying so-called epithelial cells (podocytes). These pedicels, like interlaced fingers, are about 1 to 1.5 $\mu$ long, with a free terminal end and the other end attached to a narrow ridge or trabecula (0.5 to 2$\mu$ wide) connecting to the main mass of the overlying podocyte (covering cell). The trabeculae tend to run parallel to each other about 1.5 to 2 $\mu$ apart, and give rise bilaterally to the pedicels at a frequency of about 3 to 5 per $\mu$ of edge. In no case does the main mass of the podocyte (covering cell) appear to lie directly on the surface of the definitive basement membrane (lamina densa). The typical heavily stained "basement membrane" of a histologic section, measuring, usually, 0.3 to 0.4 $\mu$ in thickness, must include the whole basement membrane complex of pedicels, lamina densa, and endothelial lamina attenuata.

Evidence will be presented to show that the basement membrane of Bowman's capsule is not invaginated in the development of the glomerulus, and that it does not form the definitive basement membrane (lamina densa) of the glomerular capillary. It will be demonstrated that Bowman's capsule terminates around the vascular pole, and that the capillary definitive basement membrane (lamina densa) is a single, distinct entity, apparently of endothelial origin, in direct continuity with the corresponding structure in the wall of the arteriole.

THE ULTRASTRUCTURE OF THE RENAL GLOMERULUS IN INTERCAPILLARY GLOMERULOSCLEROSIS. Eleanor Irvine, James F. Rinehart,*† Glenn E. Mortimore, and James Hopper, Jr., University of California School of Medicine, San Francisco, Calif.

Renal tissue, obtained by needle biopsy from patients with intercapillary glomerulosclerosis, was studied by electron microscopy. The ultrastructure of the glomerular tuft has been described by a number of investigators. It is composed of a network of capillaries, highly differentiated epithelial cells with "end-feet," and a basement membrane lying between the epithelial and endothelial cells. The endothelial cell rests upon one side of the basement membrane, and the "end-feet" of the epithelial cell terminate on or within the other.

In early intercapillary glomerulosclerosis, the first observable alteration in the renal glomerulus is in the basement membrane. This structure, which is normally

† Deceased.

of uniform thickness and homogeneous density, becomes irregularly thickened, encroaching upon the endothelial cell. Displacement of the endothelial cell narrows the capillary lumen. With progression of this process, irregular masses of material, morphologically resembling basement membrane and usually continuous with the basement membrane, become apparent within the cytoplasm of the endothelial cell. In later stages, as this material increases in amount, islands of cytoplasm are isolated by the expanding masses of basement-membrane-like material. This process continues until the nucleus and a thin rim of surrounding cytoplasm are all that remain of the former endothelial cell. There is no apparent change in the epithelial cell or in the regularity of the epithelial border of the basement membrane until replacement of the endothelial cell is well under way. There is coalescence of the epithelial "end-feet," and the resulting cytoplasmic mass becomes densely osmiophilic. Eventually the remainder of the epithelial cell cytoplasm becomes converted into an osmiophilic mass in which are remnants of mitochondria. The space occupied by the epithelial cell is gradually diminished by the expanding mass of basement-membrane-like material. The end result is a mass of structureless material in which fragments of mitochondria and nuclei are surrounded by a thin rim of cytoplasm.

Studies are in progress to further elucidate this process and to determine the similarities and dissimilarities in this and in other destructive lesions of the glomerular tuft.

BLOCKING OF UROKON $I^{131}$ CLEARANCE BY PROBENECID. R. Dominguez* and Robert B. Adams, St. Luke's Hospital, Cleveland, Ohio.

Urokon $I^{131}$ and creatinine, in an isotonic NaCl solution, were injected by constant intravenous infusion in dogs under pentobarbital anesthesia. Urine was collected from ureteral cannulas every 15 minutes. Blood samples for plasma and whole blood radioactivity and for plasma creatinine were obtained every ½ hour. Whole blood activity was recorded continuously by means of an arteriovenous shunt placed in front of a shielded scintillation counter connected to a rate meter and an automatic recorder. After a 1-hour control period, 500 mg. of probenecid was injected in five equal doses 1 minute apart. Urine and blood samples were obtained before dosage for endogenous creatinine values. Prior to probenecid, the clearances of urokon $I^{131}$ and exogenous creatinine were about equal. After probenecid, the urokon $I^{131}$ clearance became about 23 per cent of the simultaneous creatinine clearance. Three fourths of the urokon put out in the urine is therefore excreted by a tubular process which can be blocked by probenecid. At the end of the experiment, radioactivity was determined in the stomach, stomach contents, liver, small intestine, colon, spleen, left leaf of the diaphragm, thyroid gland, and kidneys. The activity per gram of spleen and muscle was used as background. The urine urokon $I^{131}$ excreted in 4 hours varied between 70 and 89 per cent of the whole amount recovered. The volume of distribution computed from the formula $V = \frac{\text{dose minus amount recovered}}{\text{final plasma concentration}}$ in per cent of body weight was, on an average, 23 for urokon (9 observations) and 65 for creatinine (6 observations). The net activity of the kidney mass, expressed in terms of the final urine concentration, varied between 0.018 and 0.054 ml./gm. (average 0.03). The dead space of the kidney, estimated by this method, amounts to about 3 ml. of urine per 100 gm. of kidney.

OBSERVATIONS ON THE PATHOGENESIS OF CLINICAL AND EXPERIMENTAL GLOMERULONEPHRITIS.† Robert C. Mellors* and Louis G. Ortega, Sloan-Kettering Institute, Memorial Center, New York, N.Y.

The present work, utilizing a microprecipitin staining reaction applicable to tissue

† See also this issue, pp. 455–499.

sections, establishes that gamma globulins are localized in the glomerular lesions in so-called lipid nephrosis of childhood and in various types of glomerulonephritis occurring at all stages, whereas gamma globulins are not detected in normal control kidneys or in kidneys that are the site of a variety of suppurative, degenerative, neoplastic, or other pathologic processes (excluding secondary amyloidosis and periarteritis nodosa). If to these observations are added nearly identical ones which we have made on two types of experimental glomerulonephritis, the one type being induced in the rabbit by repeated injections of foreign protein and the other being induced in the rat by injections of nephrotoxic serum, then a primary requisite for the allergic pathogenesis of glomerulonephritis has been generally demonstrated, i.e., the glomerular localization of gamma globulins. The analytical method of procedure in this work can be used in future studies, under specified conditions, to identify renal-localized antigens, notably those of microbial origin, and to investigate whether the localized gamma globulins are specific antibodies against such antigens.

CONCENTRATION OF BLOOD, CARCASS AND LIVER LIPIDS IN EXPERIMENTAL NEPHROSIS IN RATS, AND AFTER INJECTION OF ETHIONINE. Donald B. Hackel* and Walter Heymann, Western Reserve University, School of Medicine, Cleveland, Ohio.

Previous studies from this laboratory have shown that the hyperlipemia which develops in rats after the intravenous injection of rabbit anti-rat kidney serum is dependent on the presence of the kidneys and the liver. The present studies were designed to clarify further the pathogenesis of nephrotic hyperlipemia.

Fifty-nine rats with long-standing hyperlipemia due to chronic renal disease were studied. The concentration of lipids in the carcass of 28 of these rats (excluding the liver) gave levels in the same range as those found in 17 healthy control rats. This finding does not support the view that the hyperlipemia in the nephrotic animals is due to a shift of lipids from the tissues to the blood stream. Analysis of the livers of the nephrotic animals, however, revealed a liver lipid content that was significantly below that of the normal control rats.

When the rats with long-standing hyperlipemia were injected intravenously with dl-ethionine in concentrations over 10 mg./100 gm. of body weight/day, there was a precipitous decline in the concentration of total lipids and cholesterol in the blood. In these same animals, it was found that the liver lipid content was now within the normal range, and was significantly higher than the values in the untreated nephrotic rats. These findings support the hypothesis that hyperlipemia in the animals with nephrotoxic renal disease may be due to an inability of the liver to take up plasma lipids at a normal rate.

THE EFFECTS OF DIPAXIN ON EXPERIMENTAL NEPHRITIS IN RABBITS. Jerome Kleinerman,* Cleveland City Hospital, Cleveland, Ohio.

In the investigation of the mechanism by which heparin blocks the production of experimental nephritis, it seemed pertinent to study the effects of another anticoagulant. Dipaxin, a powerful coumarin derivative was used. Nephritis was produced in rabbits by the intravenous injection of anti-rabbit-kidney-duck sera. The optimum dose of dipaxin was determined in preliminary studies and was designed to keep the prothrombin time severely depressed below the control value. Animals were divided into three groups: (a) positive control group, given only nephrotoxic serum; (b) negative control group, given only dipaxin; (c) experimental group, given both dipaxin and nephrotic serum. Daily urine volume and semiquantitative urine proteins were done. Kidney sections were studied by hematoxylin and eosin and PAS stains. Dipaxin appeared to have no inhibitory effect on the production of experimental nephritis in rabbits. This would indicate that the mechanism of heparin inhibition is by some means other than the prevention of thrombosis and subsequent necrosis in the affected glomerular capillaries.

HISTOCHEMICAL CHANGES IN RENAL INFARCTS. Thomas L. Gavan and Nathan Kaufman,* Cleveland City Hospital, Cleveland, Ohio.

Renal infarcts were produced in albino rats by ligating the left renal artery after dissection of the perinephric fat. The right kidney was undisturbed and served as a control. Animals were sacrificed in groups of four, at intervals of 1, 2, 4, 8, 12, 18, 24, 36, and 48 hours after ligation. Portions of each kidney were fixed in 10 per cent neutral formalin, absolute alcohol, and cold acetone-alcohol mixture. The sequential tissue changes were studied using the following procedures: the periodic acid-Schiff reaction, the Feulgen reaction, Sudan IV stain, hematoxylin and eosin stain, and the calcium-cobalt methods for alkaline phosphatase and 5-nucleotidase.

5-Nucleotidase activity of the proximal convoluted tubules was first noticed to be decreased 2 hours after ligation. This activity continued to decrease so that after 48 hours no detectable enzymatic activity was seen, except in a narrow subcapsular zone. Although the same sequence was observed in alkaline phosphatase activity, the initial decrease was not seen until 4 hours after ligation and a trace of activity remained after 48 hours. The narrow subcapsular zone of preserved enzymatic activity was thought to be due to an adequate blood supply to this region via the capsular vessels. It was of interest that fatty degeneration of the tubular epithelium was first noted only after there was marked impairment in the activity of these enzymes.

Evidence of nuclear degeneration was first seen 4 hours after ligation in Feulgen stained sections. When the PAS technique was used, a PAS positive material was present in abundance along the luminal borders of the proximal convoluted tubular epithelium. At 2 hours after ligation there was a decrease in this material which corresponded with the first evidence of cloudy swelling noted in sections stained with hematoxylin and eosin.

REVERSIBLE AND IRREVERSIBLE GLOMERULAR LESIONS IN TOXEMIA OF PREGNANCY. Conrad L. Pirani,* Victor E. Pollak, John B. Nettles, Robert M. Kark, and Robert C. Muehrcke, University of Illinois, College of Medicine, Chicago, Ill.

The development of a safe technique for percutaneous needle biopsies of the kidney has permitted us to study the kidneys of women ill with toxemia of pregnancy. Biopsies were done on 30 women during pregnancy and in approximately half of them, biopsies were repeated 2 to 4 months after confinement. Clinically, all these patients presented various degrees of the three cardinal features of pre-eclampsia: hypertension, edema, and proteinuria occurring in the majority of cases during the third trimester of pregnancy. Only one case of true eclampsia was studied.

According to the histologic findings the patients were divided into five groups. In the first and largest group, the glomerular lesions were considered characteristic and consisted of edema and swelling, rather than thickening of the basement membrane. The glomerular tufts were large and bloodless, with swollen endothelial cells. There was no evidence of glomerular hypercellularity. On repeat biopsy after delivery, the glomeruli were entirely normal, indicating the complete reversibility of lesions of this type. In a second group, both swelling and thickening of the glomerular basement membrane were noted. A mild to moderate sclerosis of the small renal arteries also was present. These patients had pre-eclampsia which was as typical and approximately of the same severity as that observed in the first group of patients. On repeat biopsy, after delivery, these changes, except for the basement membrane swelling, were found to be irreversible. In a third group, irreversible vascular sclerosis was the predominant histologic feature. Glomerular basement membrane thickening was present in some of these cases but no swelling was observed. The fourth group, consisting of patients with mild toxemia, presented no renal changes of note. In the fifth group, patients with atypical toxemia were

included. In these cases, biopsy disclosed various renal diseases such as glomerulonephritis, pyelonephritis, and diabetic glomerulosclerosis.

This study disclosed that the majority of patients with typical toxemia of pregnancy have glomerular changes which are completely reversible within a few weeks after delivery. A smaller number of patients have lesions which were judged irreversible. Clinical and laboratory studies had failed to distinguish between these two groups. Renal biopsy, therefore, is of clinical usefulness in establishing prognosis and management. Whether or not one or more additional pregnancies with toxemia might lead to irreversible and gradually more severe vascular renal disease cannot be stated at present. Only long range studies of this type may clarify this problem.

MEMBRANOUS GLOMERULONEPHRITIS: ANALYSIS OF SEVEN CASES DIAGNOSED BY NEEDLE BIOPSY OF THE KIDNEY. John S. Howe* and Alvin E. Parrish, Veterans Administration Hospital, Washington, D.C.

In our series of 224 needle biopsies of the kidney performed since November, 1951, we have diagnosed 7 cases histologically as membranous glomerulonephritis. All 7 cases clinically showed the nephrotic syndrome. In 3 cases serial biopsies were performed and one case was necropsied. Our findings are in agreement with Allen and others who hold that membranous glomerulonephritis is a clinical and pathologic entity. All 7 cases showed characteristic morphologic changes in the needle biopsy specimen, readily distinguished from other and diverse renal lesions associated with the nephrotic syndrome. The diagnosis of membranous glomerulonephritis was often suspected clinically, but differentiation from the nephrotic syndrome associated with other lesions was successfully made only by biopsy. Two cases treated with steroid therapy responded favorably.

KIDNEY BIOPSY CORRELATIONS WITH HYPERTENSION. Sheldon C. Sommers,* Arnold S. Relman, and Reginald H. Smithwick, Massachusetts Memorial Hospitals, Boston, Mass.

Re-examination of approximately 1,900 surgical specimens of kidney, including 1,611 renal biopsy specimens taken at sympathectomy operations for hypertension, has provided material for investigating relationships of renal abnormalities to hypertension. Renal vascular lesions were characteristically focal rather than uniform except in the later stages. Transitional cases made possible the preparation of a synthetic pathogenesis of the renal arteriolar changes in essential hypertension, including stages of contraction, edema, hypertrophy, and fibrosis with hyalinization of degenerated arterioles. In 15 hypertensive cases the vascular abnormalities in renal biopsy specimens were insignificant, and in 18 surgical specimens fibrinoid material was deposited in arterioles, indicating a malignant nephrosclerosis. Localization of the fibrinoid by imbibition was suggested. Other types of renal disease were observed, of which chronic pyelonephritis, seen in 216 cases, was the most frequent. Reliability of the pathologic diagnoses was controlled in 200 cases, by comparing the original diagnoses of other pathologists, and by rechecking observations made in this study. Diagnoses correlated closely in 80 per cent and 87 per cent of cases, respectively.

NEEDLE BIOPSY OF KIDNEY IN MALIGNANT HYPERTENSION. John S. Howe,* Edward M. Rehak, Alvin E. Parrish, and Edward D. Freis, Veterans Administration Hospital, Washington, D.C.

Many reports have indicated the discrepancy between malignant hypertension as judged by clinical criteria and malignant nephrosclerosis diagnosed at necropsy by morphologic criteria. Recently the problem has become of practical importance in

evaluating the results of drug therapy in hypertension. Needle biopsy of the kidney seemed to offer a means of throwing some light on this problem. Accordingly, needle biopsy of the kidney was performed in a group of hypertensive patients over a period of 4 years. The patients were carefully evaluated clinically and functionally. Fourteen cases were diagnosed as malignant nephrosclerosis on histologic criteria from needle biopsy specimens. A large group of hypertensive patients with various other types of renal disease, as established by biopsy, served as a control group. The discrepancies between clinical and biopsy diagnoses are tabulated and discussed.

All cases diagnosed histologically as malignant nephrosclerosis showed characteristic staining of arteries and arterioles by Alcian blue, while other types of renal disease gave no reaction. This confirms an observation by McManus and may possibly be useful as an early test for malignant hypertension.

Patients in our series with histologic lesions of malignant nephrosclerosis have lived as long as 6 to 12 months. This seems to disprove the idea that these lesions are of terminal occurrence.

PRODUCTION OF AORTIC RUPTURE IN RATS FED PURIFIED DIETS AND BETA-AMINOPROPIONITRILE.† J. J. Lalich,* University of Wisconsin Medical School, Madison, Wis.

Some of the factors operating in the production of aortic rupture in growing rats which are fed crude sweet pea (*Lathyrus odoratus*) meal have not been fully evaluated. That other dietary constituents besides crude sweet peas are implicated is suggested by the partial protection which casein exerts against the production of skeletal deformities and an inability to produce aortic rupture in one half or more of the rats unless the casein concentration is less than 10 per cent. Sprague-Dawley rats of either sex weighing 39 to 55 gm. were used in this study. Similar diets were fed to these animals except for variations in BAPN, the B vitamins, and some amino acids. Four control and twelve test diets containing similar quantities of casein, cerelose, salt mixture, and corn oil were fed to 97 weanling rats up to 49 days. The test diets contained, in addition, BAPN and added supplements of an amino acid mixed with certain B vitamins. Aortic rupture occurred in 4 of 51 test rats in 8 assays when 1.9 gm./kg. of BAPN was fed with one of the following: dl-tryptophan, l-arginine, glycine, dl-alanine, and l-glutamic acid. In 4 assays when 8 gm./kg. of dl-methionine alone or mixed with an excess of certain B vitamins and 1.7 gm./kg. of BAPN were fed, aortic rupture occurred in 14 of 24 rats and aortic aneurysms in 6 of 10 other rats which survived. None of the amino acids and vitamin mixtures fed with BAPN prevented the development of skeletal deformities. The incidence of aortic rupture, however, could be modified by the amino acid which is fed with BAPN. Dl-methionine predisposed to aortic rupture more than any other amino acid tested thus far, when fed with BAPN to young rats which are receiving 12 per cent casein in the diet.

THE EFFECT OF HYPERCHOLESTEROLEMIA UPON INTIMAL REPAIR OF THE AORTA OF THE RABBIT FOLLOWING EXPERIMENTAL TRAUMA.‡ John T. Prior* and William H. Hartmann, State University of New York, Upstate Medical Center, Syracuse, N.Y.

A group of rabbits was subjected to mechanically induced aortic intimal trauma and maintained on a hypercholesterolemic diet for periods of 2 days up to 8½ months following the intimal injury. The injured segment disclosed increased permeability to cholesterol, and 14 days following trauma a plaque composed of lipid material and dense connective tissue was produced. This lesion was histologically

† Supported by United States Public Health Service Grant A538 (C5) from the National Institutes of Health.

‡ See also this issue, pp. 417–431.

identical with the human atherosclerotic plaques as seen in coronary arteries and the aorta. This experimentally produced lesion subsequently was characterized by necrosis and inhibition of connective tissue, so that 46 days after injury the spontaneous and traumatically induced plaques were structurally similar. Reparative connective tissue was not impressive nor was significant dystrophic calcification within the spontaneous and traumatic lesions over long periods, i.e., up to 8½ months after trauma. This inhibitory effect of cholesterol upon connective tissue repair may in part explain why the histologic counterpart of the advanced human atherosclerotic lesion cannot be duplicated by cholesterol administration. A brief review of the results of other combinations of vascular trauma and hypercholesterolemia is presented.

A Study of Carotenoids: Colored Lipids Which Infiltrate the Human Aorta. David H. Blankenhorn and David G. Freiman,* University of Cincinnati, College of Medicine, Cincinnati, Ohio, and Beth Israel Hospital, Boston, Mass.

Carotenoid pigments are found in the human aorta where they contribute to the yellow color of atheromatous plaques. They are unsaturated paraffins ($C_{40}H_{56}$) which are known to originate exclusively from the diet. A study of their distribution in 40 aortas provides information as to the manner in which lipids formed outside this vessel may infiltrate its wall. We have found that the gross display of yellow color does not accurately represent the carotenoid content found upon extraction and assay. Extensive lesions which are fibrotic, ulcerated, or calcified appear grayish white despite the fact that they contain carotenoids in amounts equal to or greater than those found in yellow lesions.

Our study has shown that up to a 250-fold parallel increase in carotenoid and cholesterol content per square centimeter is encountered when comparing an ulcerated calcified plaque with an adjacent uninvolved area. Accordingly, an infiltration of carotenoids must have occurred as cholesterol accumulated. This finding suggests that atherosclerotic plaques remain permeable to serum lipids even after extensive fibrosis and ulceration have occurred. In addition, this study demonstrates that a lipid common in the human diet, a constituent of normal serum, can infiltrate the aortic wall under conditions encountered in the usual circumstances of life.

Lipid Deposits in the Aortic Cusps of the Mitral Valve. J. F. A. McManus* and Brian K. Montgomery, University of Alabama Medical Center, Birmingham, Ala.

Two hundred hearts have been examined for lipid deposits in the aortic cusps of the mitral valve. The results have been charted from diagrams of the distribution of lipid. There is a relatively consistent distribution of lipid in a band 1 cm. or so wide which has its upper margin at the attachment of the valve cusp to the ring of the mitral valve. This is best seen from the ventricular surface. The distribution and amount of lipid is reported in 200 hearts from various ages. The importance of this lipid deposit in relation to atherosclerosis and some of its debatable features, such as vascularization and metachromasia, are discussed.

Pulmonary Hypertension and Arteriosclerosis in Rabbits.† Robert M. O'Neal, Wilbur A. Thomas, and Kyu Taik Lee, Washington University School of Medicine, St. Louis, Mo.

Arteriosclerotic lesions of the small pulmonary arteries have been produced in rabbits by the repeated intravenous injections of blood clots. We have confirmed these results. It is important to determine whether these arteriosclerotic lesions result directly from the presence of the thrombi or indirectly from pulmonary

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hemodynamic alterations (such as hypertension) induced by the obstructing thrombi. An experiment was performed to determine the importance of pulmonary hypertension in the pathogenesis of these lesions. Twenty rabbits were given repeated intravenous injections of plastic beads (lucite). The plastic was not absorbed and produced little or no tissue reaction when lodged in the pulmonary arteries. An average of 1,972 mg. of the beads was given to each rabbit. The average survival time after the first injection was 192 days. The hearts were dissected and weighed to obtain a ratio of the weight of the right ventricle to that of the left ventricle. This ratio was used as an objective, rough guide to the degree of pulmonary hypertension. A series of 29 control rabbits was studied also.

Microscopic examination of the lungs revealed that no pulmonary arterial lesions were produced in rabbits that received intravenous injections of plastic beads. The number of obstructing bodies in the pulmonary arteries, the degree of right ventricular hypertrophy, and the duration of the obstruction were greater in the rabbits receiving beads than in most other experiments in which pulmonary arteriosclerosis has been produced by the intravenous injection of blood clots. The conclusion is reached that the arteriosclerotic lesions produced by thrombo-embolism in rabbits are not simply the result of pulmonary hypertension or other hemodynamic alteration associated with obstruction. It seems likely that these arteriosclerotic lesions are actually organized thrombi.

THE NATURE OF DIFFUSE INTRA-ARTERIOLAR AND CAPILLARY OCCLUSION IN PATIENTS WITH CARCINOMA WITH SPECIAL REFERENCE TO THE SO-CALLED GENERALIZED INTRAVASCULAR COAGULATION SYNDROME.† Edwin R. Fisher* and William F. Baird, University of Pittsburgh, School of Medicine, and Veterans Administration Hospital, Pittsburgh, Pa.

The occurrence of such vascular and cardiac lesions as migratory phlebitis, purpura, and verrucal endocardiosis (indeterminate or non-bacterial endocarditis) in patients with carcinoma, particularly of the pancreas, stomach, lung, and gallbladder, is well recognized. However, the presence of disseminated intra-arteriolar and capillary lesions in such patients is less appreciated and apparently more infrequent. Because these latter vascular alterations have been purported to represent fibrin thrombi, such cases have been included as examples of the so-called intravascular coagulation syndrome. This designation has been applied for a similar reason to such obstetrical accidents as abruptio placentae, shock, necrosis of the renal cortices, pituitary body and liver, and to certain types of fatal infantile enteritis and incompatible blood transfusions. The occurrence of thrombocytopenia, prolongation of prothrombin time, fibrinogenopenia, and fibrinolysin activity have been considered to account for such clinical manifestations as shock, a hemorrhagic diathesis, acute renal failure, and pseudo-membranous colitis. In patients with carcinoma, intravascular coagulation has been ascribed to the neoplastic elaboration of thromboplastin, mucins, proteolytic enzymes, and to products of tumor necrosis all of which might be considered thrombogenic from experimental evidence. Yet such a supposition appears teleologic in the light of the results obtained in this study.

Tinctorial studies performed on two examples of diffuse intra-arteriolar and capillary occlusion associated with carcinoma revealed that, unlike fibrin, the occlusive material was markedly resistant to tryptic digestion and exhibited pronounced argyrophilia. In this regard the material behaved identically to control tissues containing fibrinoid and in conformity with our previous results and with those of others as characteristic of fibrinoid. Similar material was observed within the walls of involved vessels and in some instances various stages in the development of arteriolar and capillary occlusion could be discerned. Although fibrin also could be identified

† This article will appear in a subsequent issue of *The American Journal of Pathology*.

in some of the lesions, its quantity and location indicated that its deposition was dependent upon previous intramural and endothelial damage. In addition, the valvular vegetations encountered in one example with verrucal endocardiosis also were comprised principally of fibrinoid, a concept proposed previously.

The nature and appearance of the arteriolar and capillary lesions in these patients with carcinoma are similar to those of similar lesions encountered in thrombotic thrombocytopenic purpura, acute idiopathic thrombocytopenia, and infantile enteritis, and, as has been reported, for the generalized Shwartzman reaction produced in rabbits. In addition, some of the clinical and hematologic manifestations of the so-called generalized intravascular coagulation syndrome prevail in these instances. Such analogies based upon the nature of the fundamental lesion encountered implicate an immuno-allergic reaction in the pathogenesis of the arteriolar and capillary alterations found in some patients with carcinoma. Such a concept does not refute the existence of intravascular coagulation in such instances but minimizes the significance of such a concept in the pathogenesis of the fundamental lesions. These findings indicate the need for further tinctorial and morphologic studies in other examples of the so-called intravascular coagulation syndrome.

FACTORS IN PATHOGENESIS OF NECROTIZING VASCULAR DISEASE. Simon Koletsky,* Western Reserve University, Cleveland, Ohio.

Renal arterial ligation in the rat is followed within a few days by the development of more or less widespread necrotizing vascular disease, especially polyarteritis nodosa. This technique provides evidence that neither hypertension nor uremia are significant factors in the pathogenesis of the vascular necrosis. The lesions occur in the absence of expansion of extracellular fluid space. They are apparently not related to infection. No satisfactory evidence is available that their development is associated with a state of hypersensitivity. The vascular disease is diminished in frequency but not abolished by total adrenalectomy.

The injury to the kidney is the primary pathogenic factor in the production of vascular necrosis. This might operate through production by the kidney of a necrotizing agent elaborated elsewhere. At present it appears more likely that some form of renal dysfunction, resulting from the injury, plays a significant rôle and that this dysfunction may be related to altered electrolyte metabolism.

STUDIES ON THE CAPILLARY BED OF GUINEA-PIGS ADAPTED TO LOWER ATMOSPHERIC PRESSURE.† Enrique Valdivia, University of Wisconsin Medical School, Madison, Wis.

The total capillary bed in striated muscle of guinea-pigs born at high altitude has been reported to be greater than that of guinea-pigs born at sea level. The measurements of the total capillary bed were made on cross sections of thigh and leg muscles, previously perfused with India ink and embedded in gelatin. The capillaries per square millimeter were counted, and the capillaries around individual fibers were also counted to determine the variations in distribution. To evaluate the total capillary bed in animals adapted to lower atmospheric pressure, 8 guinea-pigs were studied after living for 3 months at a simulated altitude of 18,000 feet with only temporary exposures to sea level atmospheric pressure for cleaning and feeding. The total capillary bed in these test animals is 20 to 30 per cent greater than the total capillary bed of control guinea-pigs of the same age, weight, and sex. The capillaries also were found to be more numerous in sections from lungs, brain, striated muscle, fat, and kidneys, indicating that there is an increase of the functional capillary bed in these organs.

Values for hemoglobin and the red blood cells in animals adapted to simulated

† Supported by the Muscular Dystrophy Associations of America, Inc.

high altitude are higher than those of guinea-pigs born at high altitude. The adrenal gland-body weight ratio is also increased in all the guinea-pigs living at an altitude of 18,000 with only temporary exposures to sea level atmospheric pressure.

RETINAL MICRO-ANEURYSMS, HUMAN AND EXPERIMENTAL. J. M. B. Bloodworth, Jr.,* and G. J. Hamwi, Ohio State University, College of Medicine, Columbus, Ohio.

Recent work with flat preparations of the human retina stained by the Hotchkiss-McManus technique, and the positive periodic acid-Schiff (PAS) reaction of micro-aneurysms have stimulated an interest in mucopolysaccharide metabolism. Our studies show that human retinal micro-aneurysms are expansions of capillary walls which are themselves PAS-positive. Serial sections show that the walls of micro-aneurysms — although PAS-positive — are usually quite thin. Therefore human micro-aneurysms are not usually associated with deposition of extraneous PAS-positive material. We will demonstrate the histochemical reactions of human retinal micro-aneurysms.

The production of experimental retinal micro-aneurysms has been attempted in many animals without appreciable success. The widespread use of the rabbit as an experimental animal for the production of micro-aneurysms has prompted a careful study of its retinal vessels. The rabbit retina receives its main blood supply from the choroidal vessels. A few vessels course on the inner surface of the retina near the nerve head but do not extend into the loose central portion of the retina. In the human retina many small capillaries course in the central portion and are almost completely unsupported by connective tissue stroma. It is in these almost completely unsupported and very thin-walled capillaries that micro-aneurysms occur. Similar vessels are absent in the retina of the rabbit. This anatomical fact probably explains the difficulty experienced by investigators who have attempted to produce experimental micro-aneurysms and also indicates that the rabbit is not a suitable animal for the study of experimental micro-aneurysms. The anatomy and histology of the retinal blood supply of other experimental laboratory animals will be discussed.

CORONARY ARTERIES IN FETUSES, INFANTS, AND JUVENILES. Henry D. Moon,* University of California School of Medicine, San Francisco, Calif.

The purpose of this study was to determine the frequency and nature of pathologic processes in the coronary arteries of fetuses, infants, and young individuals. Eighty-five hearts were examined—14 from fetuses varying from 3½ to 8 months' gestation; 34 from infants up to the age of 2 years; 17 from children up to the age of 10 years; and 20 from young persons up to the age of 23 years. In all instances, except for the fetuses, death occurred suddenly, usually from severe traumatic injuries. Proximal segments of coronary arteries were sectioned and stained by hematoxylin and eosin, Weigert's elastic-tissue stain; colloidal iron-periodic acid stain for mucopolysaccharides, and aldehyde-fuchsin stain. Also, from many cases frozen sections were stained for fat.

No lesions of the coronary arteries were observed in fetuses. Rupture and fragmentation of the internal elastic membrane were observed in infants within a few days after birth. This was associated with deposition of acid mucopolysaccharide. There were subsequent intimal fibrosis and regeneration of the internal elastic membrane. In other instances, these processes had occurred repeatedly. The degree of intimal fibrosis in infants several months old was marked and indicated that this process had occurred at a rapid rate. In older children and in young adults there was an apparent decrease in the rate of intimal fibrosis. There was no significant deposition of lipid preceding or during the development of lesions of the internal elastic membrane or intima.

These processes of degeneration and regeneration of the internal elastic mem-

brane, of deposition of mucopolysaccharide, and of intimal fibrosis in the coronary arteries of infants and young individuals are identical with the early non-lipid phases of adult arteriosclerosis.

THE ETIOLOGY OF ENDOCARDIAL FIBRO-ELASTOSIS. B. Black-Schaffer,* University of Cincinnati College of Medicine, Cincinnati, Ohio.

Infantile endocardial fibro-elastosis has been elevated to a nosologic entity. It is currently held to function as a "constrictive endocarditis" leading to cardiomegaly. Following a study of 16 instances of this lesion and the application of Laplace's law of hydrostatics to the heart, it has been concluded that the tough fibro-elastic membrane could have formed only in a markedly dilated heart, since in such an organ very little constriction is necessary to expel a normal stroke volume. Therefore, the presence of even severe fibro-elastosis would exert little if any deleterious effect. This is supported by the fact that these children frequently develop few or no signs of cardiac failure almost to the week or day of death. It is, indeed, suggested that the fibro-elastic endocardium replaces in good measure the mechanical disadvantage of the dilated heart chamber with its flattened musculi carneae and trabeculae. It is probably the economical energy release of the stretched fibro-elastic tissue, in contrast to the active contraction of muscle, which enables these hearts to achieve their enormous proportions.

Thus endocardial fibro-elastosis is conceived of as a sequel to an inability of the myocardial muscle to maintain its normal configuration rather than as a congenital dyscrasia of the endocardium. In seeking an explanation of the myocardial weakness, it was learned in 4 of the cases that the cardiomegaly was due in good measure to hyperplasia rather than hypertrophy. It is possible that such a heart, for entirely physical reasons, may represent a significantly less efficient organ than the normal or hypertrophied heart.

FUNCTIONAL AND ANATOMICAL CHANGES IN EXPERIMENTAL MITRAL STENOSIS IN THE DOG. William E. Jaques,* Merlin R. Robinson, and Albert L. Hyman, Louisiana State University School of Medicine, New Orleans, La.

A supravalvular mitral stenosis was produced in 40 mongrel dogs. The dogs were followed by cardiac catheterization, femoral artery cannulation, oxygen consumption, arteriovenous oxygen differences, and electrocardiogram. Many of the animals exhibited clinical evidence of congestive heart failure. Functional studies revealed mild degrees of pulmonary hypertension, decreased cardiac outputs, and an increased total pulmonary vascular resistance. At necropsy there was generalized chronic passive congestion. There was no appreciable change in the pulmonary vasculature in the early stages of development of congestive heart failure and pulmonary hypertension. In the animals surviving for longer periods, there was slight to moderate medial hypertrophy of the arterioles and occasional intimal fibrosis. Rarely, organizing thrombi were noted in the lesser circulation. It was possible to correlate the functional and anatomical changes in a few dogs.

The effect of an acute constriction of the mitral valve area in new dogs and dogs in various stages of the development of mitral stenosis was studied. There was more passive hyperemia and edema in the new dogs following acute constriction than in those without some previous degree of mitral stenosis.

Anatomical changes in the other organs will be mentioned and especially the high incidence of pyelonephritis. The distinction between pulmonary vascular changes in experimental mitral stenosis and *Dirofilaria immitis* infestation will be discussed.

FACTORS ASSOCIATED WITH CHANGING SEX RATIO OF MYOCARDIAL INFARCTION WITH SPECIAL REFERENCE TO THE DISPROPORTIONATE RISE IN INCIDENCE OF THE

DISEASE AMONG OLDER WOMEN. Kyu Taik Lee and Wilbur A. Thomas, Washington University School of Medicine, St. Louis, Mo.

In a previous epidemiologic study of 500 necropsied patients with acute myocardial infarction at Barnes Hospital, we found a shift in the ratio of incidence of acute myocardial infarction between the two sexes. Prior to 1940 the incidence of acute myocardial infarction in this series was twice as high among men as among women, and during the period 1940 to 1954 the incidence was as high among women as it was among men. In the current study various factors that may account for this changing ratio between the sexes are analyzed and discussed.

The most remarkable observation made is that the shift in the ratio of the incidence of acute myocardial infarction between the two sexes is due to a disproportionately sharp increase in the incidence of acute myocardial infarction among women over 60 years of age during the period 1940–1954 as compared to the corresponding incidence during the period 1910–1939. Among women, neither the average body weight nor the percentage whose weights exceeded the ideal weight range was significantly different in the two periods. Thus, it seems unlikely that changes in body weight account for the disproportionate rise in incidence among women. When the weight of kidneys is used as a rough guide to the incidence and severity of systemic hypertension during life and a comparison is made between the average weights of kidneys in the two periods, no significant differences are found in either sex. Diabetes mellitus cannot account for the sharp increase in the incidence of acute myocardial infarction among women, since the incidence of diabetes mellitus among women with acute myocardial infarction is similar in the two periods.

CONGENITAL RHABDOMYOMATOSIS OF THE HEART: CASE REPORT WITH CHEMICAL AND HISTOCHEMICAL STUDIES OF THE TUMOR POLYSACCHARIDE. Robert W. Mowry,* Roger W. Hanson, and On Ja Kim, University of Alabama Medical Center, Birmingham, Ala.

By means of a special histochemical technique, copious amounts of water-soluble, alcohol-insoluble polysaccharide were shown in the tumor cells of a recently reported case of solitary rhabdomyoma of the heart. The solubility of the polysaccharide seems to explain the difficulty that others have noted in staining glycogen in this tumor. The desirability of chemical study in future instances was emphasized. The rarity of this condition has permitted few opportunities for such studies. We wish to present a new case of congenital rhabdomyomatosis of the heart in which both chemical and histochemical studies for tumor carbohydrate were performed.

A full-term colored male infant weighing 2,700 gm. was admitted the day after birth because of persistent vomiting. Gestation and delivery had seemed normal. Examination of the heart at this time and throughout the hospital course showed no abnormalities. Signs of recurrent, eventually severe, intestinal obstruction developed and led to laparotomy on the 7th hospital day. A transverse colostomy was performed. This seemed to relieve the distention and permitted the passage of feces. Distress and regurgitation were still noticed. Poor wound healing and dehiscence complicated the postoperative course. Death occurred suddenly on the 41st hospital day. There had been no fever, cyanosis, arrhythmia, or evidence of heart failure.

At necropsy, performed 5 hours post mortem, the infant appeared malnourished and weighed 1,850 gm. The heart weighed 16 gm. In the myocardium of both ventricles and in the septum there were numerous firm white nodules measuring 1 to 5 mm. in diameter. Microscopic study showed typical rhabdomyomas with some foci of calcification in both tumors and myocardium. Extensive lobular pneumonia was present in the left lower lobe of the lung. Sections of the spleen showed a few focal collections of giant cells with eosinophilic cytoplasm similar to those previously described in a case of rhabdomyoma. No further lesions were encountered in

either the gross or microscopic study of other organs of the trunk. The brain showed no evidence of tuberous sclerosis.

Histochemical studies of the tumor nodules were performed by methods described in an earlier report by one of us (R.W.M.). Tumor cells were heavily laden with polysaccharide that was only partly soluble on exposure to water. The water-resistant polysaccharide was completely absent after diastase digestion and is presumed to be glycogen. Myocardium between the nodules of tumor contained almost no glycogen.

For microchemical studies, many tumor nodules were carefully dissected away from the intervening myocardium after dehydration in multiple changes of cold ethanol. Glycogen analyses were performed on tissue aliquots heated in 30 per cent KOH at 100°C. for 90 minutes. On the basis of dry weight after ethanol extraction, the average content of glycogen in the tumor was 19 per cent; tumor free myocardium, 1.5 per cent. This carbohydrate can be described as an anthrone-reactive alkali-stable polysaccharide. Analysis of the dilute-trichloracetic acid (TCA) extractable fraction compared with the total $H_2SO_4$-extractable carbohydrate showed that all of the tumor glycogen is of the TCA-extractable type. The possible significance of these findings will be discussed.

## Read by Title

Effect of Prednisone upon Gastric Wound Healing. L. M. Cramer and E. M. Nadel,* National Institutes of Health, Bethesda, Md.

To assess the effect of prednisone ("Meticorten") upon wound healing, an incised and sutured gastric wound was studied in pair-fed and prednisone-treated C57BL male mice. Wound bursting strength and histologic appearance were studied daily for 14 days postoperatively. In untreated mice the bursting strength curve was similar to that reported for the untreated rat. The curve for the prednisone-treated animals was similar to the control with the exception of the first postoperative day, when the wound in prednisone-treated animals appeared less able to withstand internal pressure. In the prednisone-treated animals fewer inflammatory cells, less edema, and relatively fewer fibroblasts were seen on the first postoperative day. By the fifth postoperative day granulation tissue was evident in both groups. However, there was less granulation tissue, looser connective tissue stroma, and plumper fibroblasts in animals receiving prednisone. By the ninth postoperative day, all wounds had healed. However, the amount of granulation tissue and the degree of scarring and deformity appeared markedly less in the prednisone-treated group. Thus, prednisone modified the histologic response of healing in the stomach, but did not significantly alter or weaken the strength of the wound after the first postoperative day as measured by bursting pressure. Mucosal and serosal healing was not delayed after prednisone, and the decrease in the connective tissue response made for a less bulky and less deforming scar.

Occlusion of a Coronary Artery. Béla Halpert,* Ethel E. Erickson,* and Ferenc Györkey, Veterans Administration Hospital and Baylor University College of Medicine, Houston, Texas.

Infarction or extensive scarring of the myocardium due to occlusion of a coronary artery was observed in 195 of 1,800 male veteran patients examined after death. Of the patients, 1,344 were white and 456 were Negro. In 4 (3 Negro) patients the age was not available. Of these and of 139 (31 Negro) in the third decade of life, none died of coronary occlusion. Of 233 (60 Negro) in the fourth decade, 13 (3 Negro) died of coronary occlusion; of the 205 (52 Negro) in the fifth decade, 26 (3 Negro); of the 604 (152 Negro) in the sixth decade, 75 (15 Negro); of the 500 (152 Negro) in the seventh decade, 69 (9 Negro); and of the 115 (6 Negro)

in the eighth decade or over, 12 died of coronary occlusion. Occlusion of a coronary artery occurred twice as frequently in white (12 per cent) as in Negro (6 per cent) subjects. The left ventricle was involved in 177, the right ventricle or atrium in 6, and both ventricles were involved in 12 instances. Among the contributory causes considered: nephrosclerosis was encountered in all; cardiac hypertrophy in 178 with the heart weighing over 450 gm. in 134; and obesity in 73. Of the 195 patients, 22 lived from 3 to 20 years with clinical evidence of myocardial damage and gross or microscopic evidence of old or recent infarction of the myocardium; 13 survived two previous attacks; and 42 one previous attack. Of the remaining 118 patients, 21 survived the first attack from 3 months to 1 year and 97 died of the first attack immediately or within 3 months.

NEUROLOGIC SYMPTOMS PRODUCED IN RATS BY BIS-β-(CYANO-ETHYL) AMINE. Henrik A. Hartmann and Joseph J. Lalich,* University of Wisconsin Medical School, Madison, Wis.

Hind limb weakness and paralysis has been observed in rats fed sweet pea (*Lathyrus odoratus*) meal. This has been attributed to deformities of the vertebral column. While testing BAPN, the toxic factor in sweet peas, and other analogues, it was noted that bis-β-(cyano-ethyl) amine also produced distinct neurologic symptoms. The experiment was repeated in 12 animals by feeding 2.6 gm. of bis-β-(cyano-ethyl) amine per kg. of diet. Marked nervous symptoms were observed while the weight gain was only slightly less than that in the 12 control animals. The neurologic symptoms were characterized by intermittent extensor spasms of the neck muscles, general ataxia, purposeless hyperactivity, and muscle weakness. (Motion pictures of the various functional changes were taken.) The symptoms were noted in 3 animals after 3 weeks, in 7 animals after 4 weeks, and after 7 weeks all animals were similarly affected.

After 8 and 16 weeks the animals were sacrificed. Under sodium pentobarbital anesthesia, the brains were fixed immediately by intracarotid perfusion of 10 per cent formalin. Sections were taken from cerebrum, cerebellum, and spinal cord. They were stained by thionine and by hematoxylin and eosin. The most conspicuous histologic changes were observed in the anterior motor horn neurons of the spinal cords. There was a general swelling of the nerve cells to two to three times their normal size. The Nissl substance had undergone chromatolysis whereas some of the neurofibrils remained. In many cells the nuclei were disintegrated indicating necrosis. The axon of the necrotic cells showed coiling, thickening, and basophilic degeneration. These changes which were observed uniformly in all the experimental animals were attributed to a direct neurotoxic effect and not to deformities of the vertebral columns.

THE PATHOLOGY OF BRUCELLA SUIS OSTEOMYELITIS. Leo Lowbeer,* Hillcrest Medical Center, Tulsa, Okla.

Ten years ago a preliminary report was given before this Association on the histopathology of a case of *Brucella suis* infection, based on bone- and liver biopsies. The patient expired 4 years later, and a post-mortem examination revealed extensive caseating brucellotic osteomylitis involving the entire spinal column, sacrum, ilium, scapula, ribs, and right hip joint, with cold abscesses and sinus tracts. The bone lesions in the ilium and scapula, recognized radiologically and biopsied 4 years before death, had neither progressed nor healed. By contrast all liver granulomas seen in a biopsy 3 years before death had disappeared. *Brucella suis* was cultured from numerous lesions.

Microscopically, innumerable old brucellotic granulomas were found in the affected bones, with extensive central caseous necrosis, surrounded by fibrous capsules, with little cellular activity at the periphery. No tuberculoid nodules were

seen, no true epithelioid cells and, rarely, bizarre giant cells. In the vertebrae the lesions had a tendency to involve and destroy the intervertebral disks. Reactive bone formation around the granulomas was generally present. In addition to these granulomas with necrotic centers, many bone infarcts were found in their vicinity, involving previously non-infected cancellous bone, and caused by either vascular occlusions, toxicity of the pathogenic organisms, or allergic phenomena. An old cortical perforation of the scapula, observed radiologically 4 years before death, had completely healed by scarring, but the primary focus in the cancellous bone had remained stationary, with a thick wall of scar tissue surrounding an old brucellotic abscess. In the right hip joint extensive destruction of cartilage by brucellotic granulation tissue was found. Several encapsulated caseous foci were found in the spleen and mediastinal lymph nodes as well as in the thickened left pleura which 3 years before death had been the seat of acute serous pleurisy. There were small inflammatory infiltrates of mononuclear cells in lungs and heart muscle. No granulomas were found in the liver, whereas a random biopsy 3 years before death had shown many granulomas in each microscopic field.

Whereas the microscopic appearance of the brucellotic granuloma does not appear to be altogether specific for brucellosis and varies with the strain involved, it bears little resemblance to that of tubercles or gummas. In the early phases acidophilic histiocytes with round vesicular nuclei are commonly observed. In later phases of *Brucella suis* infection, caseous necrosis of toxic or allergic, but not of vascular, etiology occurs in the center and fibrous encapsulation in the periphery. A variety of small mononuclear cells remain at the periphery such as lymphocytes, plasma cells and fibrocytes. Giant cells are infrequent and bizarre. The granulation tissue contains many capillaries.

The simultaneous destruction and formation of bone in and around brucellotic osteomyelitis in its active phase, and the exquisite tendency to encapsulation of chronic lesions, causing them to become stationary by preventing their disappearance or healing, appear to be characteristic for the brucellotic process. Destruction and repair are simultaneous. This is reflected in the chronicity of the brucellotic infection. The tendency to recurrence is caused by the intracellular parasitism of the Brucella organisms, as well as by the stationary character of the brucellotic granuloma; viable Brucella organisms may at any time invade the blood stream and colonize elsewhere to cause new lesions.

HEMODYNAMICS AND THE SITUATION OF ATHEROSCLEROTIC PLAQUES. J. F. A. McManus* and J. O. Morgan, University of Alabama Medical Center, Birmingham, Ala.

We have used hydrostatic pressures to fix aortas, with a manometer and a bulb of the type used for blood pressure determinations. When the aorta is filled with formalin at the systolic pressure present during life, the yellow atheromatous plaques can be observed externally to thin out and be visible through the media. According to the pressure range from 0 to systolic, the thinning of the media can be directly produced, being most marked at the highest pressure. The customary microscopic picture of the wavy internal elastic lamina is replaced in aortas fixed at physiologic pressure by a thin linear arrangement of elastic tissue. The internal plaque which protrudes into the lumen of the aorta in the unfixed state can be seen to thin out the media under the normal blood pressure. In regard to the metachromatic ground substance, the accumulation of this material internal to the atherosclerotic plaque is much less in amount or hardly increased at all when the aorta is fixed at systolic pressure.

These findings re-emphasize the necessity for an understanding and application of physiologic conditions when dynamic structures are being studied.

FIBRINOID: DERIVATION FROM AUTOLYZED SMOOTH MUSCLE AND INJECTION INTO THE RENAL ARTERY PRODUCING AN EMBOLIC FORM. E. E. Muirhead,* Earnest Booth, and P. O'B. Montgomery,* University of Texas Southwestern Medical School, Dallas, Texas.

The authors have proposed the origin of vascular "fibrinoid" from altered smooth muscle of the media. The migration of such "fibrinoid" into the vascular lumen and its subsequent lodgment in capillaries has also been proposed. The present experiments may be considered related to these concepts.

I. Fibrinoid from smooth muscle autolyzed in the test tube. Sterile smooth muscle from the stomach and/or colon was allowed to autolyze at room temperature for 12 to 24 hours while suspended in a buffered phosphate solution of pH 7.4, then finely dispersed in a Waring blendor. Grossly and upon application on slides, this material yielded the staining and histochemical characteristics of vascular "fibrinoid." It was eosinophilic, intensely red with Mallory's aniline blue or Masson's trichrome stains, yellow with the van Gieson stain, purple with the phosphotungstic acid hematoxylin stain, and slightly metachromatic with toluidine blue. It gave positive histochemical results with the following procedures: periodic acid-Schiff's reagent (red-purple), oil red O (red), Nile blue sulfate (blue), Sudan black B (black), free potassium (black), free carbonyl groups (purple), protein bound sulfhydryl groups (red).

II. Injection into renal artery of dog. About 40 gm. of autolyzed muscle in 1,000 ml. of buffer was passed through gauze of diminishing porosity until particles capable of passing a 23 gauge needle were obtained. The kidney was delivered under anesthesia and 30 ml. of the suspension was injected into the renal artery. The kidney was returned to its bed and removed at 3 to 24 hours. After formalin fixation, regular and frozen sections using the procedures in section I were prepared. The "fibrinoid" material was observed in the interlobular arteries, capillaries of the glomeruli, and isolated tubules. It had the same characteristics as obtained *in vitro* except for minor variations. It resembled the "fibrinoid" observed in a variety of conditions.

HEPARINOCYTES IN ATHEROGENESIS. O. J. Pollak,* Kent General Hospital, Dover, Del.

Interplay of plasmatic dyscolloidity, hypo-albuminemia, osmotic pressure changes, and histamine release results in increased permeability of swollen intimal endothelium, leaking of plasma into the vessal wall, and subintimal edema. Normally, there are but few mast cells in the human aorta. Their number is increased in edematous sectors of the aorta. Patchy edema is seen on microscopic study of grossly normal vessels of persons who died in shock. Aortic mast cells are especially numerous in subjects who died in shock during post-prandial periods. Human aortic mast cells are of endothelial origin. These cells are apparently mobilized to release heparin for breakdown of plasma lipoproteins which were carried into the vessel wall in the course of reaction to stress.

THE INCIDENCE OF CARCINOMA OF THE THYROID GLAND AT NECROPSY. Yosef Leon Tiber, Nathan L. Morgenstern, and Nathan B. Friedman,* Cedars of Lebanon Hospital and University of Southern California School of Medicine, Los Angeles, Calif.

Over thirty carcinomas of the thyroid gland were found at necropsy in a series of approximately 2,000 necropsies. Metastases were demonstrated in over one half of the cases and the carcinoma was responsible for the patient's death in more than one third of the series. Half of the carcinomas which resulted in the deaths of the patients simulated carcinoma of the lung both clinically and pathologically. The significance of these findings will be discussed.